

***A Phase II Trial of Neoadjuvant Enoblituzumab (MGA271) in Men with  
Localized Intermediate- and High-Risk Prostate Cancer***

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## SYNOPSIS

Title	A Phase II Trial of Neoadjuvant Enoblituzumab (MGA271) in Men with Localized Intermediate- and High-Risk Prostate Cancer
Institution	Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD
PI(s)	Emmanuel S. Antonarakis, MD (PI), Eugene Shenderov, MD DPhil (Co-PI).
Sponsor	MacroGenics, Inc. 9704 Medical Center Drive Rockville, MD 20850
IRB #	IRB00103776
Study agent	Enoblituzumab (also referred to as MGA271)
Phase	Phase II
Indication	Men with intermediate and high-risk localized prostate cancer, prior to radical prostatectomy
Target population	<p><u>Inclusion criteria</u></p> <ul style="list-style-type: none"><li>• Histologically confirmed prostate adenocarcinoma</li><li>• Clinical stage T1c–T3b, N0, M0</li><li>• Gleason sum 7-10</li><li>• At least 2 positive cores</li><li>• Prior decision to undergo radical prostatectomy</li><li>• Adult male <math>\geq 18</math> years of age</li><li>• ECOG performance status 0-1, or Karnofsky score <math>\geq 70\%</math></li><li>• Adequate kidney, liver, and bone marrow function</li><li>• Willingness to sign informed consent and adhere to study requirements</li></ul> <p><u>Exclusion criteria:</u></p> <ul style="list-style-type: none"><li>• Presence of known lymph node involvement or distant metastases</li><li>• Prior radiation, hormones, biologics, or chemotherapy for prostate cancer</li><li>• Prior immunotherapy/vaccine therapy for prostate cancer</li><li>• Prior use of experimental agents for prostate cancer</li><li>• Concomitant treatment with hormonal therapy or <math>5\alpha</math>-reductase inhibitors</li><li>• History of autoimmune disease requiring systemic immunosuppression</li><li>• History of other malignancy within 3 years</li><li>• Uncontrolled major infectious, cardiovascular, pulmonary, hematologic, or psychiatric illnesses that would make the patient a poor study candidate</li></ul>

Start date/ Duration	<p>Initial patients are expected to be entered in November 2016. With an estimated enrollment rate of 3 patients per month, accrual is expected to last 5-6 months.</p> <p>The trial was originally designed to evaluate safety and PD endpoint with 16 patients. Based on promising PSA response data prior to prostatectomy, following Enoblituzumab therapy alone, the study will enroll an additional 16 patients for a total size of 32 – amendment 1. All patients will be enrolled utilizing identical inclusion, exclusion, and treatment conditions as the first 16 patients. Expanding the trial to enroll a total of 32 patients will provide statistical power to estimate the clinical benefit of Enoblituzumab in terms of undetectable PSA level (&lt;0.1 ng/mL) 12 months following radical prostatectomy.</p>
Sample size	<p>16 evaluable patients (<i>i.e.</i> those that undergo prostatectomy)</p> <p>16 additional patients in the expansion Amendment 1 will be enrolled for a total sample size of 32 patients.</p>
Rationale	<p>Prostate cancer remains the second most common cause of cancer related death in males, killing one in every 36 American men. While advances have been made in early detection and local treatment, few treatments have been able to dramatically alter the course of disease once it progresses to subclinical or clinical metastases. In that setting, androgen deprivation therapy is considered frontline but, while many patients will experience initial tumor regression, their prostate cancer inevitably develops a castrate resistant phenotype. Recent advances in our understanding and ability to manipulate the immune system have resulted in a sea of change in the treatment of aggressive malignancies. One promising approach to metastatic disease involves treatments that use the immune system to target and destroy tumor cells. Because an anti-tumor T cell response has the ability to dynamically evolve, immunotherapy can potentially overcome the genetic diversity of aggressive and advanced cancers. Moreover, because the immune system develops memory, anti-tumor immune responses can be potentially long-lived, and long-lived responses have been reported in patients with melanoma, kidney cancer and bladder cancer. Those recent data mostly involve immune checkpoint blockade using anti-CTLA-4 or anti-PD1/PDL1; but in prostate cancer those therapies have yielded little in the way of objective responses, suggesting the need for alternative immunological approaches.</p> <p>B7-H3 is part of the B7 superfamily which includes B7-H1(PD-L1) and B7-DC(PD-L2). Although a number of studies document a role for B7-H3 in modulating the immune response, its regulation and mechanism of action are not completely clear, with literature demonstrating both T-cell stimulatory and inhibitory effects. In addition, tumor cell expression of B7-H3 has been associated with an autonomous proliferative and migratory function. Perhaps most relevant, multiple reports (as well as our unpublished data) support a correlation between B7-H3 expression and adverse pathology and clinical outcome in men with prostate cancer. Using a large series of surgical specimens in a cohort of men uncontaminated by hormonal therapy, we found that B7-H3 expression correlates with stage and is associated with biochemical and</p>

clinical progression following treatment. In addition, B7-H3 levels remain high in the presence of androgen deprivation. These studies suggest a potential role for B7-H3 in prostate cancer progression and support its use as therapeutic target in both the hormone naïve and castrate resistant state. Here we propose a neoadjuvant study to determine the anti-tumor, immunological and biological effects of B7-H3 inhibition in men with high-risk prostate cancer.

We aim (1) to investigate whether inhibition of B7-H3 via administration of Enoblituzumab is safe and feasible in the neoadjuvant setting for men with localized intermediate and high-risk prostate cancer; (2) to determine whether Enoblituzumab produces anti-tumor responses by evaluating tumor cell apoptosis in harvested prostate glands; and (3) to determine whether Enoblituzumab is immunogenic in men with localized prostate cancer by evaluating T-cell infiltration in harvested prostate glands. We hypothesize that neoadjuvant Enoblituzumab will be feasible and safe (i.e. will not interfere with subsequent prostatectomy), and will produce measurable tumor cell death and antitumor immune responses.

If this study shows significant tumor cell apoptosis and enhancement of antitumor immunity with Enoblituzumab, and administration of the study drug is feasible and safe, then future studies would aim to use Enoblituzumab in both the adjuvant and the salvage settings. For example, a trial could be designed to assess the efficacy of Enoblituzumab given before and after radical prostatectomy, using PSA recurrence as the primary endpoint. Alternatively, a trial of Enoblituzumab for patients with PSA recurrence could be designed, with metastatic progression as the primary endpoint.

## Objectives

### Primary:

- To confirm the safety and feasibility of Enoblituzumab administered using a standard dose / schedule in the neo-adjuvant setting

In the expansion cohort (Amendment 1):

- PSA<sub>0</sub> Response (Undetectable PSA level <0.1 ng/mL) at 12 months following radical prostatectomy

### Secondary:

- To quantify an anti-tumor response to Enoblituzumab
  - TUNEL staining and quantification of tumor cell apoptosis
  - Caspase 3 staining
  - FcGamma Staining (RIIA, RIIB, RIII/RIV) (based on proposed MOA)
  - Pathological response graded according to standard criteria
- To assess the immune response to Enoblituzumab
  - Quantification of CD8 T cell and CD4 T cell infiltration into the tumor / peritumoral area
  - Determine the effect of Enoblituzumab treatment on the CD8 / Treg and CD4 / Treg ratios

- To assess Gleason grade change post neoadjuvant Enoblituzumab treatment, by comparing prostatectomy Gleason sum to pre-treatment biopsy Gleason sum
- To quantify the extent of PD-L1+ cell density in the prostate from harvested prostate glands of treated patients.
- To quantify the NK cell density in tumor tissue from harvested prostate glands of patients
- To quantify tissue androgen concentration (testosterone, dihydrotestosterone) and androgen receptor (AR) protein expression in prostate specimens

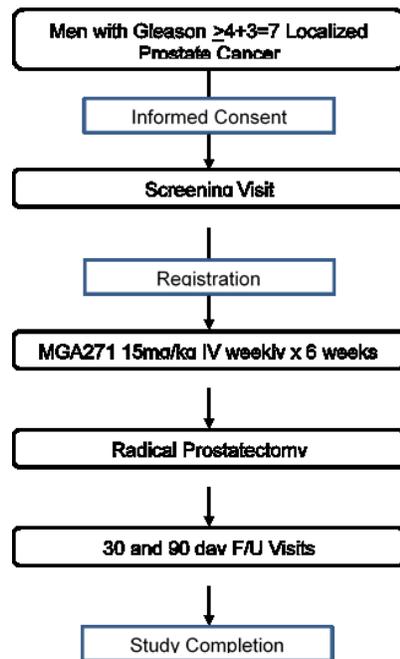
Exploratory:

- To quantify B7-H3 expression in pre-treatment and post-treatment tumor tissue and correlate with tumor cell apoptosis and time to PSA-recurrence
- To quantify PD-1, LAG3 and TIM3 in pre and post treatment tumor tissue
- Determination of Fc receptor genotype (CD16A, CD32A, CD32B)
- Global expression profiling of pre and post treatment tumor tissue using the immune NanoString immunopanel and Affy microarrays
- TCR Deep Sequencing (Adaptive Biotech) testing hypothesis that successful anti-tumor response modulates TCR repertoire in peripheral and tumor infiltrating lymphocytes.
- Quantification of antigen-spread using ProtoArray analyses

Study design

This is a single-center, single arm, open-label pilot study evaluating the safety, anti-tumor effect, and immunogenicity of neoadjuvant Enoblituzumab (MGA271) given prior to radical prostatectomy in men with intermediate and high-risk localized prostate cancer. Patients will be recruited from the outpatient Urology clinic. Eligible patients will receive Enoblituzumab at a dose of 15mg/kg IV given weekly for 6 doses beginning 50 days prior to radical prostatectomy. 14 days after the last dose of Enoblituzumab, prostate glands will be harvested at the time of radical prostatectomy, and prostate tissue will be examined for the secondary endpoints. Follow-up evaluation for adverse events will occur 30 days and 90 days after surgery. Patients will then be followed by their urologists according to standard institutional practices, but will require PSA evaluations every 3 ( $\pm 1$ ) months during year 1 and every 6 ( $\pm 2$ ) months during years 2-3.

Study Schema:



Baseline evaluations:

- Informed consent
- Medical history and physical assessment
- Review of medications
- Vital signs, including height and weight
- Performance status
- Central pathologic review of prostate core biopsies for histological diagnosis and Gleason score
- CT (If allergic to CT scan contrast, obtain MRI with contrast) and/or bone scan, if clinically indicated
- Hematology, coagulation, and chemistry laboratories
- Serum PSA measurement
- Sera for immunoassays

On weekly treatment days (days 1, 8, 15, 22, 29, 36):

- Performance status
- Interval history and focused physical assessment (including vital signs)
- Adverse events/toxicity evaluation
- Hematology and chemistry laboratories
- PBMC collection for TCR repertoire
- Fc Receptor Genotyping on day one
- Blood for PBLs (treatment days 1 and 36 only)
- Administration of Enoblituzumab 15mg/kg IV

On day 50:

- Medical history and focused physical assessment (including vital signs)
- Review of medications
- Performance status
- Adverse events/toxicity evaluation
- Hematology and chemistry laboratories
- Serum PSA
- Sera for immunoassays
- PBMC collection for TCR repertoire
- Radical prostatectomy
- Pathologic review of surgical specimen according to standard procedures
- Evaluation of harvested prostate gland for tumor cell apoptosis and CD8<sup>+</sup> T cell infiltration and other secondary endpoints

Post-operative day 30 and day 90:

- Performance status
- Adverse events/toxicity evaluation
- Hematology and chemistry laboratories
- PSA every 3 months (post-op year 1), then every 6 months (post-op years 2-3)
- Sera for immunoassays
- PBMC collection for TCR repertoire
- Blood for PBLs (Post-op day 30 only)

Criteria for evaluation

Primary endpoint:

- Frequency, type, and severity of adverse events

In expansion (Amendment 1):

- PSA<sub>0</sub> Response (Undetectable PSA level <0.1 ng/mL) at 12 months following radical prostatectomy

Secondary endpoints:

- Mean levels of apoptotic markers (TUNEL staining, caspase 3 staining, and FcGamma staining) and proliferation markers (Ki-67) in prostate tumor specimens of treated patients
- Mean CD8<sup>+</sup> T cell density in tumor tissue from harvested prostate glands of patients
- To quantify the extent of PD-L1<sup>+</sup> cell density in the prostate from harvested prostate glands of treated patients
- Mean T<sub>reg</sub> density in tumor tissue from harvested prostate glands of patients
- Mean CD8<sup>+</sup>/T<sub>reg</sub> and CD4<sup>+</sup>/T<sub>reg</sub> ratios in tumor tissue from harvested prostate glands of patients
- Mean CD4<sup>+</sup> T cell density in tumor tissue from harvested prostate glands of patients
- Mean NK cell density in tumor tissue from harvested prostate glands of patients

- Proportion of treated patients with detectable Enoblituzumab in prostate tumor specimens(fresh frozen section)
- Proportion of pathological complete responses (pCR) in each treatment group
- Proportion of patients achieving a PSA response rate (PSA <0.1 ng/mL) at 3 months after prostatectomy
- Median time-to-PSA-recurrence (PSA  $\geq$ 0.2 ng/mL) after radical prostatectomy
- Median PSA decrease prior to radical prostatectomy
- To assess Gleason grade change post neoadjuvant Enoblituzumab treatment, by comparing prostatectomy Gleason sum to pre-treatment biopsy Gleason sum

#### Statistical methods

Our primary objective(s) is to characterize safety, tolerability, and feasibility of treatment of men with Enoblituzumab in the neoadjuvant setting as well as obtain an estimate of PSA<sub>0</sub> response rate 12 months following radical prostatectomy. The trial will monitor toxicity and safety, as well as feasibility with respect to its impact on subsequent surgery. The secondary objective will be to evaluate an anti-tumor effect consistent with the agent's proposed mechanism of action (MOA), antibody-dependent cellular cytotoxicity (ADCC). Tumor cell death will be quantified by Caspase staining, and post-treatment apoptotic index compared with that from the pre-treatment biopsy. Sample size is driven by detecting a pharmacodynamic effect of the agent based on the key secondary endpoint of tumor cell death as well as in the expansion cohort to obtain an estimate of PSA<sub>0</sub> response. A biologically meaningful treatment effect will be deemed to be one where the tumor cell apoptosis is two-fold higher after Enoblituzumab therapy. Previous study showed that baseline apoptosis, measured by Caspase staining, is 0.06 +/- 0.08 (Mean +/-SD) for Gleason 4 and 0.2 ± 0.2 for Gleason 3 (Kim et al 2016). Based on this data, we assume that the coefficient of variation of tumor cell death measure is 1.2 and the correlations between the measures pre- and post-treatment is moderate at 0.4, 16 subjects will provide 88% power to detect a 2-fold increase in apoptotic tumor cells after the treatment compared to baseline, using a one-sided paired t-test with significance level 0.05. Other endpoints will be exploratory in nature, and quantified using descriptive statistics.

For the expansion cohort (amendment 1):

Our primary efficacy endpoint with 32 patients will be to estimate the clinical benefit of neoadjuvant Enoblituzumab in terms of undetectable PSA level (<0.1 ng/mL) at 12 months following radical prostatectomy. There are currently no approved neoadjuvant treatments for high risk localized prostate cancer and we believe future larger neoadjuvant trials can be better designed in high risk patients if we can estimate the benefit as per the below table showing the 90% confidence interval.

Observed N with undetectable PSA	Observed rate	90% CI
0	0	0% - 7.8%
1	3.1%	0.7% - 12.9%
2	6.2%	2.1% - 17.2%
3	9.4%	3.8% - 21.3%

**Safety analysis** Frequency, types, and grades of adverse events in each treatment group will be measured using the NCI Common Toxicity Criteria version 4.0, and will be summarized using descriptive statistics. Formal safety assessments will be performed from the time of first administration of Enoblituzumab until the 90th postoperative day.

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**Summary of changes for Protocol Amendment 1 version 1.5:**

The trial was originally designed to evaluate safety and PD endpoint with 16 patients. Based on promising PSA response data prior to prostatectomy, following Enoblituzumab therapy alone, the study will enroll an additional 16 patients for a total size of 32 – Amendment 1.

All patients will be enrolled utilizing identical inclusion, exclusion, and treatment conditions as the first 16 patients. Expanding the trial to enroll a total of 32 patients will provide statistical power to estimate the clinical benefit of Enoblituzumab in terms of undetectable PSA level (<0.1 ng/mL) 12 months following radical prostatectomy.

Section	Changes:
Synopsis	<p><b>Start date/ Duration:</b>                      The trial was originally designed to evaluate safety and PD endpoint with 16 patients. Based on promising PSA response data prior to prostatectomy, following Enoblituzumab therapy alone, the study will enroll an additional 16 patients for a total size of 32 – amendment 1. All patients will be enrolled utilizing identical inclusion, exclusion, and treatment conditions as the first 16 patients. Expanding the trial to enroll a total of 32 patients will provide statistical power to estimate the clinical benefit of Enoblituzumab in terms of undetectable PSA level (&lt;0.1 ng/mL) 12 months following radical prostatectomy.</p> <p><b>Sample size:</b>                      16 additional patients in the expansion Amendment 1 will be enrolled for a total sample size of 32 patients.</p> <p><b>Objectives:</b>                      Primary: In the expansion cohort (Amendment 1):                      •PSA<sub>0</sub> Response (Undetectable PSA level &lt;0.1 ng/mL) at 12 months following radical prostatectomy.                      Secondary:                      •To assess Gleason grade change post neoadjuvant Enoblituzumab treatment, by comparing prostatectomy Gleason sum to pre-treatment biopsy Gleason sum                      •To quantify the extent of PD-L1+ cell density in the prostate from harvested prostate glands of treated patients.</p> <p>Criteria for evaluation:                      In expansion (Amendment 1):                      •PSA<sub>0</sub> Response (Undetectable PSA level &lt;0.1 ng/mL) at 12 months following radical prostatectomy                      •Median PSA decrease prior to radical prostatectomy                      •To assess Gleason grade change post neoadjuvant Enoblituzumab treatment, by comparing prostatectomy Gleason sum to pre-treatment biopsy Gleason sum</p> <p>Statistical methods:                      For the expansion cohort (amendment 1):                      Our primary efficacy endpoint with 32 patients will be to estimate the clinical benefit of neoadjuvant Enoblituzumab in terms of undetectable PSA level (&lt;0.1 ng/mL) at 12 months following radical prostatectomy. There are currently no approved neoadjuvant treatments for high risk localized prostate cancer and we believe future larger neoadjuvant</p>

	<p>trials can be better designed in high risk patients if we can estimate the benefit as per the below table showing the 90% confidence interval.</p> <table border="1"> <thead> <tr> <th>Observed N with undetectable PSA</th> <th>Observed rate</th> <th>90% CI</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0</td> <td>0% - 7.8%</td> </tr> <tr> <td>1</td> <td>3.1%</td> <td>0.7% - 12.9%</td> </tr> <tr> <td>2</td> <td>6.2%</td> <td>2.1% - 17.2%</td> </tr> <tr> <td>3</td> <td>9.4%</td> <td>3.8% - 21.3%</td> </tr> </tbody> </table>	Observed N with undetectable PSA	Observed rate	90% CI	0	0	0% - 7.8%	1	3.1%	0.7% - 12.9%	2	6.2%	2.1% - 17.2%	3	9.4%	3.8% - 21.3%
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<b>1.1 Primary Objectives</b>	<p>In expansion cohort (amendment 1):</p> <ul style="list-style-type: none"> <li>•To estimate PSA<sub>0</sub> Response Rate (Undetectable PSA level &lt;0.1 ng/mL) at 12 months following radical prostatectomy</li> </ul>															
<b>1.2 Secondary Objectives</b>	<ul style="list-style-type: none"> <li>•To quantify the extent of PD-L1+ cell density in the prostate from harvested prostate glands of treated patients.</li> <li>•To quantify the extent of NK cell infiltration into the prostate from harvested prostate glands of treated patients.</li> <li>• Enoblituzumab (MGA271) intraprostatic drug distribution in treated patients.</li> <li>• To assess Gleason grade change post neoadjuvant Enoblituzumab treatment, comparing prostatectomy Gleason sum to pre-treatment biopsy Gleason sum</li> </ul>															
<b>1.3 Exploratory Objectives</b>	<ul style="list-style-type: none"> <li>•IHC analyses of CD137, CD16 and/or CD107A across potential immune infiltrate following Enoblituzumab (based on proposed MoA)</li> <li>•Tissue androgen concentrations</li> <li>•Androgen receptor (AR) quantification</li> <li>•Global expression profiling of pre and post treatment tumor tissue using single cell RNA sequencing, the immune NanoString immunopanel and/or Affy microarrays</li> </ul>															
<b>Table 1 Study Calendar</b>	Footnote C: These labs should be collected prior to treatment.															
<b>8.2.2 In expansion cohort (amendment 1)</b>	<ul style="list-style-type: none"> <li>•All subjects receiving at least one dose of the study drug will be assessed for PSA<sub>0</sub> Response Rate (Undetectable PSA level &lt;0.1 ng/mL) at 12 months following radical prostatectomy</li> </ul>															
<b>8.3.4 PD-L1 expression</b>	<p>PD-L1 expression in prostate tumor specimens will be assessed by IHC in the primary core specimens (pre-treatment) and in the prostatectomy surgical specimens (post-treatment). This endpoint will be expressed as the mean staining percentage of PD-L1 in tumor tissue.</p>															
<b>8.3.7</b>	<p>Mean NK cell density in tumor tissue from harvested prostate glands of patients.</p> <p>The method for quantifying NK cell density from harvested prostate tissue is as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue.</p>															
<b>8.3.19</b>	<p>In expansion cohort (Amendment 1): assessment of Gleason grade change (prostatectomy Gleason sum vs. core biopsy Gleason sum) post neoadjuvant Enoblituzumab treatment</p>															

	This will be defined by comparing highest Gleason grade from pre-treatment biopsy versus highest Gleason grade from post-treatment prostatectomy. This endpoint will be expressed as the proportion of men achieving a Gleason score change.																													
<b>8.3.20</b>	Global expression profiling of pre and post treatment tumor tissue using single cell RNA sequencing, the immune NanoString immunopanel and/or microarrays Expression profiling will be conducted as per established protocols for single cell RNA seq, Nanostring, and microarray																													
<b>8.3.21</b>	IHC analyses of CD137, CD16 and/or CD107A across potential immune infiltrate following Enoblituzumab  CD137, CD107A, and CD16 expression in prostate tumor specimens will be assessed by IHC in the prostatectomy surgical specimens (post-treatment). This endpoint will be expressed as the mean staining percentage of each of these in in tumor tissue.																													
<b>10.1 Study Design and Sample Size</b>	In Amendment 1, the study was expended to enroll an additional 16 patients for a total of 32 patients to continue evaluating safety and better estimate the clinical benefit of Enoblituzumab in terms of undetectable PSA level (<0.1 ng/mL) at 12 months following radical prostatectomy.																													
<b>10.2 Stopping rules</b>	Not feasible if the observed number of surgical complications is: <table style="margin-left: 40px;"> <tr> <td>2</td> <td>3</td> <td>4</td> <td>5</td> <td>6</td> </tr> <tr> <td>In number of patients between:</td> <td>2 - 6</td> <td>7 - 14</td> <td>15 - 22</td> <td>23 - 30</td> <td>31 - 32</td> </tr> </table> The operating characteristics of the first 3stopping rule are shown below and are based on 5000 simulations: <table style="margin-left: 40px;"> <tr> <td>Risk of surgical complication</td> <td>0.025</td> <td>0.05</td> <td>0.10</td> <td>0.15</td> <td>0.20</td> </tr> <tr> <td>% of Time study stops</td> <td>1.1%</td> <td>5.9%</td> <td>29.2%</td> <td>57.7%</td> <td>81.4%</td> </tr> <tr> <td>Expected sample size</td> <td>31.7</td> <td>30.7</td> <td>26.4</td> <td>20.9</td> <td>15.3</td> </tr> </table>	2	3	4	5	6	In number of patients between:	2 - 6	7 - 14	15 - 22	23 - 30	31 - 32	Risk of surgical complication	0.025	0.05	0.10	0.15	0.20	% of Time study stops	1.1%	5.9%	29.2%	57.7%	81.4%	Expected sample size	31.7	30.7	26.4	20.9	15.3
2	3	4	5	6																										
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<b>10.2.1.1 For expansion Amendment 1</b>	Estimation of PSA <sub>0</sub> Response Rate (Undetectable PSA level <0.1 ng/mL) at 12 months following radical prostatectomy. Our primary efficacy endpoint will be to estimate the clinical benefit of neoadjuvant Enoblituzumab in terms of undetectable PSA level (<0.1 ng/mL) at 12 months following radical prostatectomy. The estimate and corresponding 90% confidence interval based on 32 patients have complications is listed in the table below. <table style="margin-left: 40px;"> <tr> <td>Observed N with undetectable PSA</td> <td>Observed rate</td> <td>90% CI</td> </tr> <tr> <td>0</td> <td>0</td> <td>0% - 7.8%</td> </tr> <tr> <td>1</td> <td>3.1%</td> <td>0.7% - 12.9%</td> </tr> <tr> <td>2</td> <td>6.2%</td> <td>2.1% - 17.2%</td> </tr> </table>	Observed N with undetectable PSA	Observed rate	90% CI	0	0	0% - 7.8%	1	3.1%	0.7% - 12.9%	2	6.2%	2.1% - 17.2%																	
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	3	9.4% 3.8% - 21.3%
<p><b>10.2.2 Analysis of the secondary and exploratory endpoints</b></p>	<ul style="list-style-type: none"> <li>•PD-L1+ cell density. The methods for quantifying and analyzing PD-L1 cell density will be similar to those described for CD8+ T cell infiltration. Descriptive statistics and graphical summaries will be provided</li> <li>•Mean NK cell density. The methods for quantifying and analyzing NK cell infiltration will be similar to those described for CD8+ T cell infiltration. Descriptive statistics and graphical summaries will be provided.</li> <li>•Enoblituzumab (MGA271) drug distribution. Intraprostatic Enoblituzumab staining concentrations will be summarized descriptively.</li> <li>•Gleason grade change post neoadjuvant Enoblituzumab treatment from pre-treatment biopsy. This will be calculated utilizing the highest Gleason grade from the pre-treatment biopsy and the post-treatment prostatectomy.</li> </ul>	
<p><b>APPENDIX F</b></p>	<ol style="list-style-type: none"> <li>1. Sera for Immunoassays : Please Ping “Vaccine Team” (pager 410-283-0693) and the Immune Core will be notified to pick up the sample after draw.</li> <li>2. Peripheral blood lymphocytes (PBLs): Please Ping “Vaccine Team” (pager 410-283-0693) and the Immune Core will be notified to pick up the sample after draw.</li> </ol>	

## **1. OBJECTIVES**

### **1.1 Primary Objectives**

- To evaluate the safety and tolerability of MGA271 administered weekly x 6 (2 weeks prior to prostatectomy).
- To evaluate the feasibility of administering MGA271 weekly x 6 (2 weeks prior to prostatectomy).

In expansion cohort (amendment 1):

- To estimate PSA<sub>0</sub> Response Rate (Undetectable PSA level <0.1 ng/mL) at 12 months following radical prostatectomy

### **1.2 Secondary Objectives**

- To quantify markers of apoptosis (TUNEL staining, caspase 3 staining, FcGamma staining) in prostate tumor specimens of treated patients.
- To quantify markers of cell proliferation (Ki-67) in prostate tumor specimens of treated patients.
- To quantify the extent of CD8<sup>+</sup> T cell infiltration into the prostate from harvested prostate glands of treated patients.
- To quantify the extent of PD-L1<sup>+</sup> cell density in the prostate from harvested prostate glands of treated patients.
- To quantify the extent of CD4<sup>+</sup> T cell and T<sub>reg</sub> infiltration into the prostate in prostate specimens of treated patients.
- To quantify CD8<sup>+</sup>/T<sub>reg</sub> and CD4<sup>+</sup>/T<sub>reg</sub> ratios in prostate specimens of treated patients.
- To quantify the extent of NK cell infiltration into the prostate from harvested prostate glands of treated patients.
- Enoblituzumab (MGA271) intraprostatic drug distribution in treated patients.
- To evaluate the proportion of pathological complete responses (pCR) in prostate tumor specimens of treated patients.
- To evaluate PSA response rates and the time-to-PSA-recurrence after radical prostatectomy in treated patients.
- To assess Gleason grade change post neoadjuvant Enoblituzumab treatment, comparing prostatectomy Gleason sum to pre-treatment biopsy Gleason sum

### **1.3 Exploratory Objectives**

- To quantify B7-H3 expression in pre and post treatment tumor tissue and correlate with tumor cell apoptosis and time to PSA-recurrence
- To quantify PD-L1, PD-1, LAG3 and TIM3 in pre and post treatment tumor tissue

- Determination of Fc receptor genotype (CD16A, CD32A, CD32B)
- IHC analyses of CD137, CD16 and/or CD107A across potential immune infiltrate following Enoblituzumab (based on proposed MoA)
- Tissue androgen concentrations
- Androgen receptor (AR) quantification
- Global expression profiling of pre and post treatment tumor tissue using single cell RNA sequencing, the immune NanoString immunopanel and/or microarrays
- TCR Deep Sequencing (Adaptive Biotech) testing hypothesis that successful anti-tumor response modulates TCR repertoire
- Quantification of antigen-spread using ProtoArray analyses or similar analyses

## 2. BACKGROUND AND RATIONALE

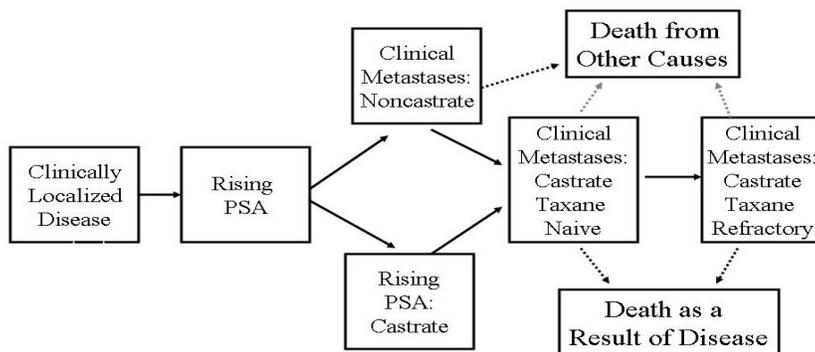
### 2.1 Disease Background

Prostate cancer is the second leading cause of cancer deaths in men. Approximately one in every six American men will be diagnosed with the disease during his lifetime (Siegel et al. *Cancer Statistics 2012*). Yet, localized prostate cancer is often curable and even metastatic disease can respond to treatment.

The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states (Figure 1). These clinical states involve the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death. Based on the extent of disease, hormonal status, and absence or presence of detectable metastases on imaging studies, the states are: localized disease, rising PSA after radiation therapy or surgery with no detectable metastases, and clinical metastases in the non-castrate or castrate settings.

Treatment of localized prostate cancer usually consists of surgery or radiation therapy or both. For patients with high-risk or locally advanced disease, a combination of radiation therapy and hormonal therapy is often used. However, even after definitive local therapy, approximately 30-50% of patients will have a local or distant recurrence (Pound et al 1999, D'Amico et al 2000). The risk of recurrence depends on multiple factors including tumor stage, tumor Gleason score, and serum PSA at the time of diagnosis. In addition, PSA doubling time at recurrence has been shown to be a powerful predictor of distant metastases and survival (Antonarakis et al 2012; Freedland et al 2007).

Figure 1 Clinical States of Prostate Cancer



Patients with metastatic prostate cancer have a median survival of 3-7 years, and most die of the disease. Treatment for metastatic disease involves surgical castration or hormonal manipulation using luteinizing hormone-releasing hormone (LHRH) agonists/antagonists, antiandrogens, or both. Although the majority of these patients initially respond to androgen deprivation therapy, all eventually progress to a state of castration-resistant prostate cancer (CRPC).

It is now accepted that CRPC is not androgen-independent and continues to rely on androgen signaling (Longo *NEJM 2010*). Owing to this new understanding, several drugs have recently emerged for the treatment of castration-resistant prostate cancer; these agents either suppress the synthesis of extragonadal androgens or target the androgen receptor directly. Enzalutamide is an inhibitor of androgen-receptor signaling that exerts its activity by binding avidly to the ligand-binding domain of the androgen receptor, competing with and displacing the natural ligands of this receptor (testosterone and dihydrotestosterone) while also inhibiting translocation of the androgen

receptor into the nucleus and impairing transcriptional activation of androgen-responsive target genes (Tran et al. *Science* 2009; Scher et al. *Lancet* 2010). Abiraterone is an inhibitor of cytochrome P450 17A1 (CYP17A1) that impairs androgen-receptor signaling by depleting adrenal and intratumoral androgens (O'Donnell et al. *Br J Cancer* 2004; Attard et al. *JCO* 2008). After studies showed improved survival with these drugs, (Scher et al. *NEJM* 2012; de Bono et al. *NEJM* 2011; Ryan et al. *NEJM* 2013) both agents were approved by the Food and Drug Administration for the treatment of metastatic castration-resistant prostate cancer.

Although enzalutamide and abiraterone represent breakthroughs in the treatment of metastatic castration-resistant prostate cancer, approximately 20 to 40% of patients have no response to these agents with respect to prostate-specific antigen (PSA) levels (i.e., they have primary resistance). Among patients who initially have a response to enzalutamide or abiraterone, virtually all eventually acquire secondary resistance. Other acceptable approaches in these men include watchful waiting until the development of symptoms, or the initiation of cytotoxic chemotherapy. In this regard, docetaxel has been shown to improve overall survival in patients with CRPC, but only by a median of 2.9 months (Tannock et al 2004; Berthold et al 2008). However, because therapies for metastatic prostate cancer are palliative and not curative, there remains an urgent need to improve the outcome of primary therapies for prostate cancer.

## 2.2 Treatment Background

### 2.2.1 Background on B7-H3

The B7 family of cell surface molecules consists of structurally related protein ligands that bind to receptors on lymphocytes and regulate immune responses. Activation of T and B lymphocytes is initiated by engagement of antigen-specific receptors, T cell antigen receptor (TcR) and membrane-bound immunoglobulin (mIg) respectively, but additional signals delivered simultaneously to members of the CD28 family of receptors by B7 ligands determine the ultimate immune response (Collins et al 2005). B7 homolog 3 (B7-H3) is a novel member of the B7 family. B7-H3 has been implicated in the delivery of both co-stimulatory and co-inhibitory signals (Hofmeyer et al 2008). The apparent contrasting activities of B7-H3 may be attributed to multiple factors. While the murine B7-H3 molecule exists as a 2-Ig form, the human counterpart has undergone gene duplication and exists primarily as a 4-Ig molecule (Steinberger et al 2004). Further, as with other members of the B7 family, B7-H3 may bind, on different cells, to multiple receptors that remain to be identified.

B7-H3 is an attractive target for tumor immunotherapy with regard to its immunological properties. Tissue expression studies have demonstrated that B7-H3 protein is not expressed in most normal tissues, rather its expression is inducible on certain antigen presenting cells (Suh et al 2003, Chapoval et al 2001) and vasculature, exists on certain endocrine tissues (most notably in the cytoplasm of epithelial cells of the adrenal cortex), and is over-expressed in a wide range of cancers (including cultured cancer stem-like cells). B7-H3 is broadly over-expressed on many malignant neoplasms, including SCCHN (MacroGenics unpublished observation); bladder cancer (MacroGenics unpublished observation, Boorjian et al 2008); prostate cancer (Zang et al 2007, Roth et al 2007, Thompson et al 2009, Chavin et al 2009), renal cell carcinoma (Crispen et al 2008), where B7-H3 is broadly expressed in tumor vasculature; ovarian cancer (Zang et al 2010); colorectal cancer (Sun et al 2010); gastric cancer (Wu et al 2006); non-small cell lung cancer (Sun et al 2006); and melanoma (Loo et al 2012).

Most notably, in prostate cancer, expression of B7-H3 is associated with metastatic behavior and poor outcome. Utilizing immuno-histochemistry, Roth and colleagues demonstrated that B7-H3 is expressed in normal prostate epithelium and is more intensely expressed by prostate cancer ([Roth et al 2007](#)). Within their series, virtually all prostate cancer expressed B7-H3, however the intensity of expression varied with cancers exhibiting more aggressive phenotypes (larger tumors, those with extra-prostatic extension) expressing higher levels of the protein. In addition, marked levels of B7-H3 expression correlated with disease progression following surgery. These results were supported by work from Zang et al. which also demonstrated increased expression of B7-H3 in the majority of prostate cancers and particularly increased expression in tumors which were non-organ confined at surgery ([Zang et al 2007](#)). In their series, strongly positive B7-H3 expression was prognostic of clinical failure and death from prostate cancer in univariate models. Further correlating with this data, high B7-H3 expression was also found to be prognostic of disease progression following salvage radiation therapy for recurrent prostate cancer after surgery ([Parker et al 2011](#)). In addition to these studies on primary prostate cancer tissue, B7-H3 protein expression has also been explored in metastasis and in prostate cancer treated with androgen deprivation. This study demonstrated B7-H3 expression in bone metastasis with a trend towards increased expression upon androgen deprivation ([Chavin et al 2009](#)). Neoadjuvant androgen deprivation did not appear to alter expression in prostatectomy tissue.

### 2.2.2 Study Agent Enoblituzumab (MGA271) Background

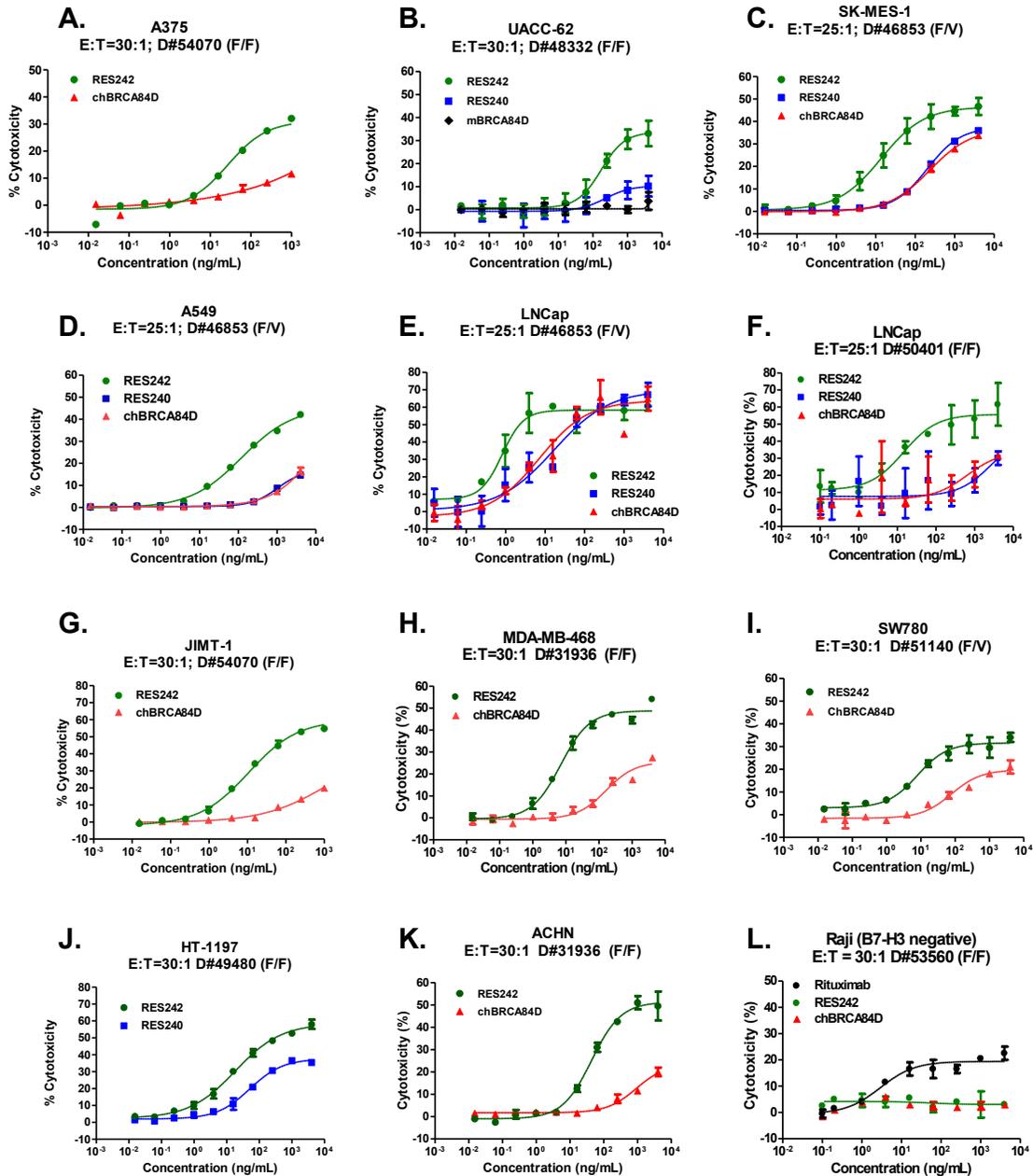
MGA271 is a humanized monoclonal antibody that binds the B7-H3 immunoligand with high affinity ([Loo et al 2012](#)). The antibody has been engineered to have enhanced binding to the activating Fc $\gamma$ R, CD16A, and especially the low affinity allele of CD16A, CD16A-158F. Since most patients carry the low-affinity allele of CD16A, the enhanced binding of the Fc-optimized version is expected to impart binding improvement that benefits the whole patient population, not just those patients who are homozygous for the high-binding allele of CD16A (valine/valine (V/V) genotype, approximately 15% of the population). MGA271 also exhibits reduced binding to the low-affinity inhibitory receptor, CD32B.

Antibody dependent cellular cytotoxicity (ADCC) has been shown to be an important mechanism of action for several monoclonal antibodies including rituximab ([Clynes et al 2000](#), [Weng et al 2003](#)) and trastuzumab ([Clynes et al 2000](#), [Musolino et al 2008](#)) and is likely an important mechanism of action for MGA271.

The ability of MGA271 (or RES242 [MGA271 with a wild-type Fc]) to mediate ADCC activity was evaluated across multiple cancer types expressing varying levels of B7-H3 as determined by flow cytometry. The cancer types tested included: melanoma (A375, UACC-62), lung cancer (SK-MES-1, A549), prostate cancer (LnCAP), breast cancer (JIMT-1, MDA-MB-468), bladder cancer (SW780, HT-1197), and renal cancer (ACHN) cell lines.

MGA271-mediated ADCC activity against all tumor lines that expressed B7-H3 at detectable levels. Furthermore, MGA271 showed enhanced ADCC potency compared to the related version of the antibody with wild type Fc domains, chBRCA84D or RES240, against all the tumor cell lines examined (see Figure 2). Consistent with the studies described above, the greatest enhancement in ADCC activity against the B7-H3 expressing prostate cancer cell line LnCAP was observed with effector populations obtained from individuals homozygous for the weak binding allele of CD16A (phenylalanine/phenylalanine [F/F]). In contrast, MGA271 did not mediate ADCC against Raji B-cell lymphoma cells, which do not express detectable cell surface B7-H3.

Figure 2 MGA271 (RES242) Mediates ADCC Activity Across a Spectrum of Cancer Types



The ability of MGA271 to mediate ADCC was evaluated on B7-H3 positive melanoma (A&B), lung (C&D), prostate (E&F), breast (G&H), bladder (I&J), and renal cancer (K) cell lines, as well as the B7-H3 negative Raji B cell lymphoma line (L). MGA271 activity was compared to the indicated control molecules: humanized BRCA84D (hBRCA84D); chimeric BRCA84D (chBRCA84D), and mouse BRCA84D (mBRCA84D). Resting human PBMC from independent healthy donor were utilized and the effector cell to target cell (E:T) ratio is

indicated in each panel. The percent cytotoxicity, as determined by lactate dehydrogenase (LDH) release, was calculated.

A series of in vivo xenograft tumor efficacy studies (RR-RND-MGA271-10-1003) were conducted to identify tumor cell lines that are sensitive to MGA271 as a single agent, establish dose response profiles for the antitumor activity of MGA271, and identify cancer types that exhibit sensitivity to MGA271. Weekly administration of MGA271 resulted in significant inhibition of growth of a variety of human tumor xenografts representing bladder, gastric, lung, melanoma, prostate, and kidney cancer. MGA271 exhibited antitumor activity when administered approximately 1 week after tumor cell implantation or after tumors were allowed to become fully established (approximately 3 weeks after implantation when tumors were ~300 mm<sup>3</sup> in volume). See MGA271 Investigator's Brochure for additional details.

### 2.2.3 Safety of MGA271

#### 2.2.3.1 Nonclinical Data

A repeat dose Good Laboratory Practice (GLP) toxicology study was conducted in cynomolgus monkeys to determine the potential toxicity and toxicokinetics of MGA271 when administered weekly for 4 weeks via intravenous (IV) infusion at doses of 1, 10, 30, or 150 mg/kg and to evaluate recovery from any effects of the MGA271 over a dose-free period of at least 8 weeks. In this study, MGA271 was well tolerated at all dose levels evaluated. There were no test article-related mortalities and no test article-related changes in clinical observations, food consumption, body weight, clinical pathology parameters, gross pathology, and organ weights. The no observed adverse effect level (NOAEL) was considered to be 150 mg/kg. The detailed report of pathology, laboratory and IHC findings in the cynomolgus monkeys from the repeat-dose GLP toxicology study can be found in the **IND 111005 - IB Version 7**.

In IHC studies, MGA271 was shown to recognize epithelial elements in the normal human adrenal cortex, and in tissue distribution studies MGA271 was found to distribute to the adrenal cortex in cynomolgus monkeys treated with a 150 mg/kg dose. However, no changes in hormone parameters were observed in cynomolgus monkeys treated with MGA271.

#### 2.2.3.2 Clinical Trial Experience

A total of 160 adult patients have received MGA271 at doses up to 15 mg/kg, the maximum dose given in this study. One-hundred and forty-six of these patients received MGA271 in which MGA271 was given alone (no other chemotherapy was administered with MGA271). In other studies, 14 patients received MGA271 in combination with another drug for cancer treatment. To date, no person under the age of 18 has been treated with MGA271.

So far, the most important safety risk that has been identified with MGA271 is infusion-related reaction (including reactions known as cytokine release syndrome [CRS]). Infusion-related reactions are effects to a drug that may occur during or shortly after an infusion. Signs and symptoms of an infusion-related reaction may include fever, chills, nausea, vomiting, headache, muscle stiffness, rash, itching, low blood pressure, and difficulty breathing. Infusion-related reactions can be life threatening and, in rare cases, may cause death. Infusion related-reactions have occurred in a total of 37% (about 4 in 10) patients

receiving treatment with MGA271. Most of the infusion-related reactions observed in patients receiving MGA271 have been mild to moderate in severity. However, six patients receiving MGA271 (< 4% of patients) have had serious or severe infusion-related reactions. These patients, some of whom were hospitalized for these reactions, recovered after receiving treatment with steroids, antihistamines, intravenous fluids, and other medications. One patient was discontinued from the study after experiencing a moderate infusion reaction.

The following were the most common side effects that were considered related to MGA271 administration and were seen in at least 1 of 10 participants. These side effects have been generally mild or moderate.

- Infusion-related reactions (including CRS) (37%)
- Fatigue (32%)
- Nausea (18%)
- Chills (14%)
- Vomiting (13%)

A total of 18 patients (11% or 1 in 10 patients) have experienced serious or severe side effects that were considered related to MGA271. Serious or severe side effects that occurred in 3 or more patients were infusion related reactions (6 patients, described above) and hyponatremia (which occurred in 3 patients).

There have not been any deaths considered related to the use of MGA271.

## 2.3 Rationale

### 2.3.1 Rationale for conducting the study

Prostate cancer remains the second most common cause of cancer related death in males, killing one in every 36 American men. While advances have been made in early detection and local treatment, few treatments have been able to dramatically alter the course of disease once it progresses to subclinical or clinical metastases. In that setting, androgen deprivation therapy is considered frontline but, while many patients will experience initial tumor regression, their prostate cancer inevitably develops a castrate resistant phenotype. Recent advances in our understanding and ability to manipulate the immune system have resulted in a sea of change in the treatment of aggressive malignancies. One promising approach to metastatic disease involves treatments that use the immune system to target and destroy tumor cells. Because an anti-tumor T cell response has the ability to dynamically evolve, immunotherapy can potentially overcome the genetic diversity of aggressive and advanced cancers. Moreover, because the immune system develops memory, anti-tumor immune responses can be potentially long-lived, and long-lived responses have been reported in patients with melanoma, kidney cancer and bladder cancer. Those recent data mostly involve immune checkpoint blockade using anti-CTLA-4 or anti-PD1/PDL1; but in prostate cancer those therapies have yielded little in the way of objective responses, suggesting the need for alternative immunological approaches.

B7-H3 is part of the B7 superfamily which includes B7-H1 (PD-L1) and B7-DC (PD-L2). Although a number of studies document a role for B7-H3 in modulating the immune response, its regulation and mechanism of action are not completely clear, with literature

demonstrating both T-cell stimulatory and inhibitory effects. In addition, tumor cell expression of B7-H3 has been associated with an autonomous proliferative and migratory function. Perhaps most relevant, multiple reports (as well as our unpublished data) support a correlation between B7-H3 expression and adverse pathology and clinical outcome in men with prostate cancer. Using a large series of surgical specimens in a cohort of men uncontaminated by hormonal therapy, we found that B7-H3 expression correlates with stage and is associated with biochemical and clinical progression following treatment. In addition, B7-H3 levels remain high in the presence of androgen deprivation. These studies suggest a potential role for B7-H3 in prostate cancer progression and support its use as therapeutic target in both the hormone naïve and castrate resistant state. Here we propose a neoadjuvant study to determine the anti-tumor, immunological and biological effects of B7-H3 inhibition in men with high-risk prostate cancer.

We aim (1) to investigate whether inhibition of B7-H3 via administration of MGA271 is safe and feasible in the neoadjuvant setting; (2) to determine whether MGA271 produces anti-tumor responses by evaluating tumor cell apoptosis in harvested prostate glands; and (3) to determine whether MGA271 triggers an immune response in men with localized prostate cancer by evaluating T-cell infiltration in harvested prostate glands. We hypothesize that neoadjuvant MGA271 will be feasible and safe, and will produce measurable antitumor immune responses.

If this study shows significant tumor cell apoptosis and enhancement of antitumor immunity with MGA271, and administration of the study drug is feasible and safe, then future studies would aim to use MGA271 in both the adjuvant and the salvage settings. For example, a trial could be designed to assess the efficacy of MGA271 given before and after radical prostatectomy, using PSA recurrence as the primary endpoint. Alternatively, a trial of MGA271 for patients with PSA recurrence could be designed, with metastatic progression as the primary endpoint.

### 2.3.2 *Rationale for dosage selection*

In the CP-MGA271-01 Phase I study, dose escalation was carried out from 0.01 mg/kg to 15 mg/kg without a DLT observed at any dose level. As 15 mg/kg was the highest protocol specified dose, 15 mg/kg was determined as the maximum administered dose (MAD), and that dose carried into the expansion phase of that study. The safety profile of MGA271 based on a total of 51 patients exposed to MGA271 demonstrated that the majority of AEs were mild (CTCAE Grade 1 or 2), with toxicities manageable by standard medical therapy. One patient has discontinued MGA271 for a drug related toxicity that was considered to be a drug-related SAE. (See **Investigator's Brochure version 8** for a more detailed description of the safety profile from Study CP-MGA271-01).

Preliminary PK modeling indicates that MGA271 administered at doses below 5 mg/kg IV on a weekly schedule follows a target-mediated drug disposition model. At doses above 5 mg/kg, exposure increased approximately linearly with the dose (See **Investigator's Brochure version 5, Table 9**). At doses above 5 mg/kg, linear two-compartment model with clearance,  $CL=0.201$  L/day; central volume,  $V_c=3.22$  L; inter-compartment clearance,  $Q=0.700$  L/day; and peripheral volume,  $V_p= 3.68$  L, provided adequate description of the observed data. The distribution half-life (i.e., the initial rapid distribution to tissues [ $T_{1/2\alpha}$ ]) and the terminal half-life [ $T_{1/2\beta}$ ] were estimated at 1.6 days and 25 days, respectively.

### 2.3.3 Rationale for immunobiologic endpoints

The primary efficacy goal of this study is to assess direct antitumor effects in prostate tumors after administering the protocol therapy consistent with the agent's proposed mechanism of action (MOA), antibody-dependent cellular cytotoxicity (ADCC). To this end, we will measure tumor apoptosis and proliferation using validated markers such as activated caspase 3 (Marcelli et al 2000) and Ki-67 (Rubin et al 2002). These will be assessed by immunohistochemical staining of formalin-fixed tissue samples. Ki-67 is a nuclear antigen that is found in proliferating cells, whose expression correlates with the degree of apoptosis and cellular turnover in prostate cancer (Berges et al 1995). Apoptotic cells will also be identified histologically and quantified by the method of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL assay) (Furuya et al 1995).

In an exploratory fashion, we will also assess B7-H3 expression in pre and post treatment tumor tissue and correlate with tumor cell apoptosis and time to PSA-recurrence.

We will also assess the degree of immune infiltration into the prostate after administering the protocol therapy. In studies using the ProHA × TRAMP mouse model, the primary endpoint was hemagglutinin (HA)-specific CD8<sup>+</sup> T cell responses in harvested prostate glands (Wada et al 2009). Our previous studies have shown that the mean staining percentage for CD8<sup>+</sup> T cells in human prostate tumor tissue is 0.42% (standard deviation 0.36%, interquartile range 0.07%–0.71%). In the present study, CD8<sup>+</sup> T cell density will serve as a surrogate measure of prostate-/prostate cancer-specific T cell responses.

We will also evaluate the proportion of patients achieving a pathological complete response (pCR).

## 3. PATIENT SELECTION

### 3.1 Target Population

Subjects will include men with multifocal, Gleason 7 or greater, clinically localized prostate cancer for whom the decision has been made to perform radical prostatectomy at Johns Hopkins Hospital. Subjects will be identified and recruited through the Outpatient Clinic of the Department of Urology and from the Multidisciplinary Prostate Cancer Clinic.

### 3.2 Inclusion Criteria

To be eligible for this study, patients must meet *all* of the following criteria:

- Histologically confirmed adenocarcinoma of the prostate (clinical stage T1c–T3b, N0, M0) without involvement of lymph nodes, bone, or visceral organs
- Initial prostate biopsy is available for central pathologic review, and is confirmed to show at least 2 positive cores and a Gleason sum of  $\geq 7$
- Radical prostatectomy has been scheduled at Johns Hopkins Hospital
- Age  $\geq 18$  years
- ECOG performance status 0-1, or Karnofsky score  $\geq 70\%$  (see Appendix A)
- Adequate bone marrow, hepatic, and renal function:
  - WBC  $>3,000$  cells/mm<sup>3</sup>
  - ANC  $>1,500$  cells/mm<sup>3</sup>

- Hemoglobin >9.0 g/dL
- Platelet count >100,000 cells/mm<sup>3</sup>
- Serum creatinine <1.5 × upper limit of normal (ULN)
- Serum bilirubin <1.5 × ULN
- ALT <3 × ULN
- AST <3 × ULN
- Alkaline phosphatase <3 × ULN
- The etiology of abnormal bilirubin and transaminase levels should be evaluated prior to study entry.
- Willingness to provide written informed consent and HIPAA authorization for the release of personal health information, and the ability to comply with the study requirements (**note:** HIPAA authorization will be included in the informed consent)
- Willingness to use barrier contraception from the time of first dose of MGA271 until the time of prostatectomy.

### 3.3 Exclusion Criteria

To be eligible for this study, patients should *not* meet *any* of the following criteria:

- Presence of known lymph node involvement or distant metastases
- Other histologic types of prostate cancers such as ductal, sarcomatous, lymphoma, small cell, and neuroendocrine tumors
- Prior radiation therapy, hormonal therapy, biologic therapy, or chemotherapy for prostate cancer
- Prior immunotherapy/vaccine therapy for prostate cancer
- Prior use of experimental agents for prostate cancer
- Concomitant treatment with other hormonal therapy or 5 $\alpha$ -reductase inhibitors
- Current use of systemic corticosteroids or use of systemic corticosteroids within 4 weeks of enrollment (inhaled corticosteroids for asthma or COPD are permitted as are other non-systemic steroids such as topical corticosteroids)
- History or presence of autoimmune disease requiring systemic immunosuppression (including but not limited to: inflammatory bowel disease, systemic lupus erythematosus, vasculitis, rheumatoid arthritis, scleroderma, multiple sclerosis, hemolytic anemia, Sjögren syndrome, and sarcoidosis)
- History of malignancy within the last 3 years, with the exception of non-melanoma skin cancers and superficial bladder cancer
- Uncontrolled major active infectious, cardiovascular, pulmonary, hematologic, or psychiatric illnesses that would make the patient a poor study candidate
- Known prior or current history of HIV and/or hepatitis B/C

### 3.4 Inclusion of Minorities

Men of all races and ethnic groups will be considered for study participation. Candidates must conform to all eligibility criteria to be accepted into the study. Minority patients who meet entry criteria will be actively recruited, although the trial is not designed to measure differences in

outcomes between ethnic groups. The estimated breakdown of the target population by race and ethnicity is: 80% white/Caucasian, 15% black/African American, and 5% comprised of other ethnic minorities.

### 3.5 Prohibited Concomitant Medications

Concurrent use of other anticancer agents or therapies including other experimental treatments is not permitted. Patients may not currently be taking any other form of androgen deprivation therapy, antiandrogens, 5 $\alpha$ -reductase inhibitors, chemotherapy, radiation therapy, biologic therapy, immunosuppressive medications, or systemic corticosteroids.

Within 30 days of administration of study drug, patients should not receive vaccines including but not limited to the live rotavirus vaccine, the live BCG (bacillus Calmette-Guerin) vaccine, the live influenza vaccine, the live measles vaccine, the live mumps vaccine, the live poliovirus vaccine, the live rubella vaccine, the smallpox vaccine, the typhoid vaccine, the varicella vaccine, and the yellow fever vaccine.

Because of the potential for unknown drug-drug interactions, concurrent use of all other agents, over-the-counter medications, herbal remedies, vitamins/minerals, and alternative therapies must be documented on the case report form (CRF).

## 4. REGISTRATION AND ENROLLMENT PLAN

### 4.1 Registration Procedure

Patients who are considered candidates for the study will first be evaluated for eligibility by one of the principal investigators, co-investigators, or the research nurse. After screening for eligibility, patients who are eligible to participate in the trial must be registered with the Sidney Kimmel Comprehensive Cancer Center (SKCCC) according to the instructions below. A record of patients who fail to meet entry criteria (*i.e.*, screen failures) will also be maintained. Registration must be completed before beginning any study-related activities.

To initiate registration at SKCCC, study personnel should forward copies of the signed informed consent form (with embedded research authorization/HIPAA form), the institutional registration form, plus any required pathology information/laboratory tests to the project manager by fax or email. Once eligibility is confirmed, each subject will be assigned a unique patient study identification number. Treatment must not commence until the patient has received his identification number.

Prior to protocol enrollment and initiation of treatment, subjects must sign and date an IRB-approved consent form. Authorized study personnel should fully explain the scope of the study to each patient before obtaining informed consent. Patients should be advised of any known risks inherent in the planned treatments/procedures, any alternative treatment options, their right to withdraw from the study at any time and for any reason, and their right to privacy. When obtaining informed consent, study personnel should: **first**, confirm that the patient has received and has had time to read the informed consent form (including the research authorization/HIPAA form); **next**, confirm eligibility as defined in Sections 3.2 and 3.3 (inclusion and exclusion criteria); and **finally**, obtain dated and signed informed consent. A copy of the signed informed consent should be supplied to the project manager.

To register a patient, the following documents must be completed and faxed 410-614-7273 or emailed ([thefka1@jhmi.edu](mailto:thefka1@jhmi.edu)) to the study coordinator (Taylor Hefka):

- signed patient consent form
- institutional registration form

- copies of the prostate cancer pathology report and baseline laboratory studies including CBC with differential, liver and kidney function tests. Other materials may also be sent if considered pertinent for confirming patient eligibility.

The principal investigator and/or other authorized study personnel will then review these documents to confirm eligibility. To complete the registration process, the project manager will assign a patient study number (*i.e.*, protocol patient number). This number is unique to the patient and must be written on all data and correspondence for the patient. The project manager will also register the patient with SKCCC's Clinical Research Office (CRO).

#### **4.2 Expected Enrollment**

A total of 32 patients will be included in this study. The first patients are expected to be enrolled in February 2016, once the protocol has been approved by the IRB. With an estimated enrollment rate of 3 patients per month, accrual is expected to be completed in 5-6 months.

#### **4.3 Study Centers**

This is a single-institution study that will be conducted only at Johns Hopkins through a collaboration between the Brady Urological Institute and the Sidney Kimmel Comprehensive Cancer Center.

#### **4.4 Recruitment**

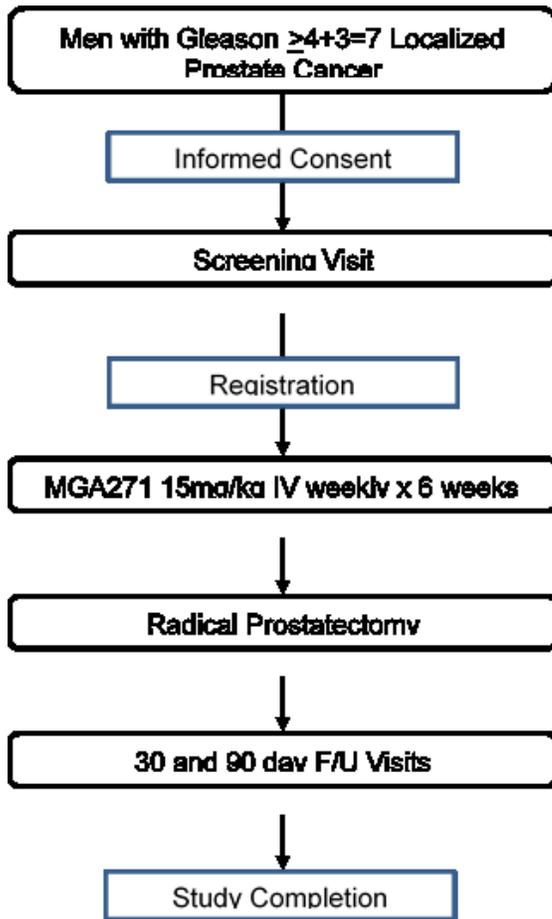
Subjects will be recruited from the outpatient facilities of the Brady Urological Institute, and from the outpatient Multidisciplinary Prostate Cancer Clinic of the SKCCC. Patients will not receive payment or reimbursement for participation. Every effort will be made to include patients of racial and ethnic minorities who fulfill the eligibility criteria.

### **5. STUDY PLAN**

#### **5.1 Overview and Schema**

This is a single-center, single arm, open-label pilot study evaluating the safety, anti-tumor effect, and immunogenicity of neoadjuvant MGA271 given prior to radical prostatectomy in men with high-risk localized prostate cancer. Patients will be recruited from the outpatient Urology clinic. Eligible patients will receive MGA271 at a dose of 15mg/kg IV given weekly for 6 doses beginning 50 days prior to radical prostatectomy. 14 days after the last dose of MGA271, prostate glands will be harvested at the time of radical prostatectomy, and prostate tissue will be examined for the secondary endpoints. Follow-up evaluation for adverse events will occur 30 days and 90 days after surgery. Patients will then be followed by their urologists according to standard institutional practices, but will require PSA evaluations every 3 ( $\pm 1$ ) months during year 1 and every 6 ( $\pm 2$ ) months during years 2-3.

#### **Study Schema:**



**Table 1 STUDY CALENDAR**

Every effort should be made to keep visits, tests, and procedures on schedule. Acceptable deviations are listed below.

	Screening Evaluation <sup>a</sup>	Treatment Days (1, 8, 15, 22, 29, 36) (+/- 1 day)	Radical Prostatectomy, Day 50 (+/- 3 days)	Follow-up, 30, and 90 Days Post-op (+/- 1 wk) <sup>h,i</sup>
Informed consent	X			
Medical history	X		X	
Review of medications	X	X	X	X
Physical assessment	X	X	X	
Vital signs	X	X	X	
Height and weight	X	X	X	
Performance status	X	X	X	X
CT and bone scan <sup>b</sup>	(X)			
Hematology labs <sup>c</sup>	X	X	X	X
Chemistry labs <sup>d</sup>	X	X	X	X
Serum PSA <sup>e</sup>	X <sup>e</sup>		X	X <sup>e</sup>
MGA271 dose		X		
Toxicity assessment	X-----X			
Surgical specimen <sup>f</sup>			X	
Pathologic review <sup>g</sup>	X		X	
Sera for Immunoassays <sup>l</sup>	X		X	X
Whole Blood (100 mL) for PBLs collection		X <sup>k</sup>		X <sup>k</sup>
B7-H3 IHC	X <sup>g</sup>		X <sup>f</sup>	
Fc Receptor Genotyping		X <sup>j</sup>		
TCR Repertoire (8.5 mL PaxGene DNA)		X <sup>j</sup>	X <sup>j</sup>	X <sup>j</sup>

- a: The screening (pre-treatment) evaluation should be conducted within 3 weeks (±4 days) of starting protocol therapy.
- b: Staging CT (if allergic to CT scan contrast, obtain MRI with contrast) and bone scans should only be performed if clinically indicated, and are not mandatory.
- c: Hematology laboratories include hemoglobin, hematocrit, white blood cell count with differential (including absolute eosinophil count), and platelets. In addition, prothrombin time (PT/INR) and activated partial thromboplastin time (APTT) should be checked as clinically indicated. These labs should be collected prior to treatment.
- d: Chemistry laboratories include sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, glucose, calcium, albumin, total protein, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, amylase, and lipase.
- e: Serum PSA should be obtained pre-treatment (at least 30 days from biopsy) and on the day of surgery, and every 3 months (±1 mo) for post-op year one then every 6 months (±2 mo) for post-op years two and three.
- f: Harvested prostate gland to be evaluated for tumor apoptosis, CD8+ T cell infiltration, as well as other secondary and exploratory endpoints.
- g: Archival prostate core biopsies to be centrally reviewed at baseline prior to study entry; radical prostatectomy specimens to be processed at Johns Hopkins according to standard procedures. Archival tissue for B7-H3 IHC testing to be sent to MacroGenics.
- h: The post-operative evaluations (30 and 90 days after prostatectomy) may take place over the telephone or in person, but patients are required to have hematology and chemistry labs collected at this time-point for safety purposes.
- i: In addition, PSA should be measured every 3 months (±1 month) in the first year after prostatectomy, and every 6

- months ( $\pm 2$  months) in the second and third years after prostatectomy.
- j: Fc Receptor Genotyping and TCR Repertoire is collected prior to treatment in one 8.5 mL PaxGene DNA collection tube. TCR Repertoire is collected on the day of surgery, and 30 and 90 days post-op.
  - k: PBLs should be collected on treatment day 1 (pre-treatment), treatment day 36 (post-treatment), and follow-up day 30 (see Appendix F)
  - l: See Appendix F for specimen collection instructions for Sera for immunoassays.

## 5.2 Screening/Pretreatment Evaluation

Before initiating any screening activities, the scope of the study should be explained to each patient. Patients should be advised of any known risks inherent in the planned procedures, any alternative treatment options, their right to withdraw from the study at any time and for any reason, and their right to privacy. After this explanation, patients should be asked to sign and date an IRB-approved informed consent form that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50).

The pretreatment/screening visit will determine patient eligibility according to the inclusion and exclusion criteria. All subjects must undergo a number of baseline evaluations as part of this screening visit, as detailed below. All of these evaluations should be conducted within  $21 \pm 4$  days of starting the protocol. This information is also summarized in the Study Calendar (Table 1).

- informed consent
- demographic information
- medical history, including review of systems
- performance status, using ECOG or Karnofsky scales (Appendix A)
- physical assessment
- vital signs: temperature, pulse, sitting blood pressure, respiratory rate
- height and weight
- current medication list, including drug allergies/adverse events
- hematological laboratories (hemoglobin, hematocrit, white blood cell count with differential [including absolute eosinophil count], platelets); coagulation profile (INR, aPTT) if clinically indicated
- serum chemistry profile (sodium, potassium, chloride, bicarbonate, urea, creatinine, glucose, calcium, albumin, total protein, bilirubin, ALT, AST, alkaline phosphatase, amylase, lipase)
- serum PSA level
- CT (If allergic to CT scan contrast, obtain MRI with contrast) and/or bone scan, only if clinically indicated (not mandatory)
- central pathologic review of prostate core biopsies
- Sera for immunoassays

After all relevant screening information is documented, registration should be finalized and appropriate documents (*i.e.*, signed informed consent, supporting source documentation for eligibility) should be faxed or emailed to the program manager.

Information on patients who do not meet eligibility criteria to participate in this study (*i.e.*, screening failures) should also be captured at the pretreatment visit.

## 5.3 Treatment Visits

Treatment initiation with MGA271 may begin at least 1 week after diagnostic biopsy granted the patient does not have any complications from biopsy. The following must be performed on treatment days (days 1, 8, 15, 22, 29, 36) ( $\pm 1$  day) with administration of MGA271.

- performance status
- review of medication list

- review of toxicity/adverse events
- height/weight
- vital signs (before, halfway through, and after 2-hr infusion)
- physical exam (focused)
- hematological laboratories and serum chemistry profile
- PBMC collection for TCR repertoire
- Whole blood for PBLs (treatment days 1 and 36 only)
- Whole blood for Fc receptor genotyping Day 1 only
- Administration of MGA271 15mg/kg IV (see section 6.3 Study Drug Administration)

#### **5.4 Radical Prostatectomy**

The following must be performed on the day of radical prostatectomy, which itself should occur 50 ( $\pm 3$ ) days after the administration of the first dose of MGA271.

- medical history
- performance status
- physical assessment, including vital signs
- review of medication list
- review of toxicity/adverse events
- hematological laboratories and serum chemistry profile
- serum PSA
- collection of prostatectomy tissue for analysis of study endpoints
- pathological processing of prostatectomy specimen according to standard procedures
- Sera for immunoassays
- PBMC collection for TCR Repertoire

#### **5.5 Follow-Up Evaluations**

Follow-up visit scheduled for 30 days and 90 days after radical prostatectomy may occur in the outpatient clinic. Required evaluations during this follow-up visits are listed below. Patients should then continue to be followed by their treating urologist according to standard institutional practices.

- performance status
- review of medication list
- review of toxicity/adverse events
- hematological laboratories and serum chemistry profile
- Serum PSA at post-operative day 90
- Sera for immunoassays
- PBMC collection for TCR Repertoire
- Whole blood for PBLs (post-op day 30 only)

Patients withdrawing from the study early because of adverse events should be followed until the adverse event has either resolved or stabilized. Reasons for premature withdrawal should be determined and documented.

In addition, patients will be required to have a structured assessment of post-operative PSA measurements for 3 years. In the first year after prostatectomy, PSA will be measured every 3 months ( $\pm 1$  mo). In the second and third years after prostatectomy, PSA will be measured every 6 months ( $\pm 2$  mo). These PSA measurements may be obtained outside of Johns Hopkins, but the results need to be made available to the study team.

## 5.6 Duration of Therapy

Participation in this study will be terminated for any of the following reasons listed below:

- the patient decides to withdraw from the study (withdrawal of consent) due to unacceptable toxicities or for any other reason
- the patient completes all of the protocol procedures and follow-up requirements
- there are adverse events that, in the judgment of the investigator, may cause severe or permanent injury or are incompatible with continuation on study
- there are major violations to the study protocol or the patient is noncompliant with treatments, as judged by the investigator
- the patient experiences concurrent illness or a change in his condition that, in the judgment of the investigator, renders him unacceptable for further treatment on study
- the patient dies
- the patient is lost to follow-up
- the study is prematurely terminated for safety or feasibility concerns or other reasons

Patients should be removed from the study when any of the above criteria are met. Because an excessive rate of withdrawals can render the study uninterpretable, unnecessary withdrawal of patients should be avoided if possible. When a patient leaves the study early, the investigator should make every effort to contact the patient and to perform the final follow-up evaluation (even by telephone interview). The reason for removal of a patient from the study, and the date of removal, must be appropriately documented.

Patients will be replaced if they are removed from the study after signing the informed consent but before undergoing radical prostatectomy. Patients receiving at least one dose of the study drug will be included in safety analyses, and those also undergoing prostatectomy will be used for the efficacy analyses.

## 6. STUDY TREATMENT

### 6.1 Description of Study Drug and Supplies

The MGA271 drug product is a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow or pale brown solution supplied at a protein concentration of 25 mg/mL in a single-use 20 mL vial containing 17 mL (425 mg) MGA271. The product is formulated in 0.95 mg/mL sodium acetate trihydrate, 0.18 mg/mL glacial acetic acid, 90 mg/mL sucrose, 0.1 mg/mL polysorbate 80 and Water for Injection, United States Pharmacopeia (USP) at a pH of 5.1.

MGA271 is supplied as a sterile aqueous solution packaged in a USP and Ph. Eur. conforming Type I borosilicate, 20 mL clear glass vial with a 20 mm FluroTec-coated 4432/50 gray butyl rubber serum stopper. The vial is sealed with a 20 mm TruEdge aluminum closure with a plastic overseal. MGA271 will be administered by IV infusion over 120 minutes. A sterile, non-pyrogenic, low-protein binding polyethersulfone (PES) 0.2 micron in-line filter administration set must be used for IV administration of MGA271. MGA271 must not be administered as an IV push or bolus.

Under no circumstances is the Investigator allowed to release these clinical supplies for use by another physician not named on Form FDA 1572 or to administer study drug to a patient who is not enrolled in this study. Study drug must be dispensed at an institution specified on Form FDA 1572.

Requests to MacroGenics, Inc for additional study drug should be made at least 2 weeks in advance.

## 6.2 Drug Preparation

### 6.2.1 General Guidelines and Precautions

The calculated dose will be administered based on the patient's actual weight at Day 1. Significant ( $\geq 10\%$ ) change in body weight from baseline should prompt recalculation of dose.

Infusion or allergic reactions may occur with the infusion of monoclonal antibodies and other protein-based therapeutics. Precautions for anaphylaxis should be observed during MGA271 administration. Supportive measures may include, but are not limited to: epinephrine, antihistamines, corticosteroids, IV fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen. Please refer to **Section 6.4.4** for specific guidelines regarding the management of infusion reactions. Supportive care measures consistent with optimal patient care will be provided throughout the study according to institutional standards.

### 6.2.2 MGA271

- Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have pale-yellow color), or there is foreign particulate matter.
- The desired amount of MGA271 should be withdrawn from the vial(s) and diluted to the appropriate final concentration with 0.9% Sodium Chloride Injection USP, according to the instructions provided in the Pharmacy Manual.
- The infusion bag containing MGA271 should be gently inverted to mix the solution. THE BAG MUST NOT BE SHAKEN; excessive agitation may cause aggregate formation.
- Discard partially used vials of MGA271
- Administration of study drug should begin immediately after preparation but no later than 6 hours after preparation (see Pharmacy Manual). If there is a delay in administration of study drug such that it will not be administered on the day of preparation, the Medical Monitor should be notified immediately. Instructions on how to proceed will be provided.

### 6.2.3 *Placebo or Control*

There will be neither placebo nor active control drug for this study.

## 6.3 **Study Drug Administration**

- Do not mix MGA271 with, or administer as an infusion with, other medicinal products.
- Administer the diluted solution over 120 minutes through an intravenous line using an infusion pump. A sterile, non-pyrogenic, low-protein binding polyethersulfone (PES) 0.2 micron in-line filter administration set must be used for IV administration of MGA271.
- Do not use non-polyolefin IV infusion bags.

## 6.4 **Potential Adverse Events and Supportive Care Measures**

### 6.4.1 *Infusion Related Reactions Including Cytokine Release Syndrome*

MGA271 is an immune modulating agent that may lead to T-cell activation and killing of the tumor cell. Activation of T cells is associated with the production of various cytokines.

Infusion reactions (including cytokine release syndrome [CRS]) associated with MGA271 administration should be managed according to the standard practice of medicine. General guidelines for the management of such reactions are provided in this section. However, severe reactions may require more intensive interventions (e.g., steroids, anti-TNF $\alpha$  antibodies, and/or IL-6 inhibitors).

Infusion related-reactions have occurred in a total of 37% (about 4 in 10) patients receiving treatment with MGA271. Most of the infusion-related reactions observed in patients receiving MGA271 have been mild to moderate in severity. However, six patients receiving MGA271 (< 4% of patients) have had serious or severe infusion-related reactions. These patients, some of whom were hospitalized for these reactions, recovered after receiving treatment with steroids, antihistamines, intravenous fluids, and other medications. One patient was discontinued from the study after experiencing a moderate infusion reaction.

Patients should be monitored closely for CRS symptomology in the infusion clinic for the first **2 hours after the end of the initial MGA271 infusion and then for 1 hour after the remaining infusions**. Patients should also be monitored closely for the development of other infusion-related reactions during the MGA271 infusion. Medications and supportive measures for the treatment of severe hypersensitivity reactions should be available for immediate use for an infusion reaction during study drug administration and may include, but are not limited to: subcutaneous (SC) epinephrine (0.3 to 0.5 mL of a 1:1000 solution), antihistamines (e.g., diphenhydramine 25 to 50 mg IV), corticosteroids (e.g., hydrocortisone 20-40 mg IV push or equivalent), IV fluids, vasopressors, oxygen, bronchodilators, and antipyretics. Resuscitation equipment and other supplies for the emergency management of an allergic/toxic reaction must be available. The patient should be treated according to the best available local practices and procedures. All supportive measures consistent with optimal patient care will be provided throughout the study according to institutional standards.

Should symptoms of fever or chills develop it may be difficult to distinguish among potential causes of the symptoms including emerging infection, or infusion reaction. Patients should be evaluated carefully for the presence of infection, with the acquisition of cultures and/or

implementation of empiric antibiotic therapy as appropriate based on the assessment of the Investigator. Please refer to **Section 6.4.4** for guidance regarding the management of infusion reactions.

#### 6.4.2 *Grading of Infusion Reactions*

Infusion reactions will be categorized as follows:

- Grade 1: mild reaction; infusion interruption not indicated, intervention not indicated; Note: although interruption in infusion is not indicated, temporary rate reduction indicated before resuming original rate, as patient tolerates (see **Section 6.4.4**);
- Grade 2: therapy or infusion interruption indicated but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs (NSAIDS), narcotics, IV fluids]; prophylactic medications indicated for  $\leq 24$  hours;
- Grade 3: prolonged (e.g., not rapidly responsive to medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates);
- Grade 4: life-threatening consequences; pressor or ventilatory support indicated;
- Grade 5: death.

The above grading scale is the CTCAE v 4.03 grading scale for CRS, which is nearly identical to the CTCAE v 4.03 grading scale for infusion reaction and allergic reaction, and therefore considered appropriate for grading all infusion reactions in this study, irrespective of the underlying mechanism of the reaction. The Sponsor's Medical Monitor or designee should be contacted immediately if questions arise concerning the grade of the reaction.

#### 6.4.3 *Premedications and Prophylaxis*

For MGA271, the following suggested guidelines (which may be modified by the investigator) are measures to be followed to avoid potential infusion reactions.

Prior to first infusion (guidelines to be followed):

- Acetaminophen 650 mg
- Diphenhydramine 50 mg or appropriate dose of equivalent H1 antagonist
- Ranitidine 300 mg or appropriate dose of equivalent H2 antagonist at the discretion of the investigator
- Hydrocortisone 20 to 40 mg (dose selected at the discretion of the Investigator)

Prior to subsequent MGA271 infusion, investigators may use the following premedications as considered indicated (suggested guidelines):

- Acetaminophen 650 mg
- Diphenhydramine 50 mg or appropriate dose of equivalent H1 antagonist
- Ranitidine 300 mg or appropriate dose of equivalent H2 antagonist
- Optional hydrocortisone 20 to 40 mg (dose selected at the discretion of the Investigator)

For subsequent MGA271 doses, patients who had infusion reactions who were not adequately or only moderately controlled with acetaminophen, diphenhydramine, or ranitidine, IV hydrocortisone at doses of 20-40 mg may be considered.

#### 6.4.4 *Management of Observed Infusion Reactions*

The following are treatment guidelines for MGA271 (which may be modified as needed by the Investigator according to the best practices of medicine) for infusion reactions.

##### 6.4.4.1 *Grade 1:*

- Slow the infusion rate by 50%.
- Monitor the patient for worsening of condition.
- Continue rate at 50% reduction and increase dose rate to the original rate by doubling the infusion rate after 30 minutes, as tolerated to the initial rate. Consideration can be given to beginning all subsequent infusions at 50% rate and increasing as tolerated.
- If a patient has an infusion reaction with MGA271, prophylactic preinfusion medications should be considered prior to all subsequent MGA271 infusions as written below.
- The following prophylactic preinfusion medications are recommended prior to future infusions of MGA271 for patients who experience Grade 1 infusion reactions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 650 mg at least 30 minutes before additional study drug administrations.

##### 6.4.4.2 *Grade 2:*

- Stop the infusion.
- Administer diphenhydramine hydrochloride 25-50 mg IV, acetaminophen 650 mg orally for fever, and oxygen and bronchodilators for mild bronchospasm.
- Resume the infusion at 50% of the prior rate once the infusion reaction has resolved or decreased to Grade 1. The rate may then be escalated to the original rate after 30 minutes, as tolerated. Consideration can be given to beginning all subsequent infusions at 50% rate and increasing as tolerated.
- Monitor for worsening condition. If symptoms recur, discontinue the infusion; no further study drug will be administered at that visit.
- For patients with Grade 2 infusion reactions despite premedication with diphenhydramine and acetaminophen, corticosteroids (hydrocortisone 20-40 mg IV or equivalent) should be considered for acute management of the event and should be added to the premedication regimen for subsequent dosing of MGA271.

##### 6.4.4.3 *Grade 3:*

- STOP THE INFUSION AND DISCONNECT THE INFUSION TUBING FROM THE PATIENT.
- TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING – ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN

- Administer diphenhydramine hydrochloride 25-50 mg IV, hydrocortisone 25-100 mg IV (or equivalent), and other medications/treatment as medically indicated. Higher doses of corticosteroids (i.e. methylprednisolone 2 -4 mg/kg IV) may also be considered for acute management.
- IV fluids, supplemental oxygen and bronchodilators should be considered as appropriate.
- If symptoms have resolved to baseline within 12 hours, a re-challenge may be considered at the next scheduled dose, with a 50% reduction of infusion rate. In addition, patients should be pre-medicated for this re-challenge and for any subsequent doses of MGA271 with the following: diphenhydramine hydrochloride 25-50 mg IV, oral acetaminophen 625 mg and hydrocortisone 25-100 mg IV. Patients who have a Grade 3 infusion reaction that does not resolve within 12 hours despite medical management should not receive further MGA271.
- Patients who experience a second Grade 3 infusion reaction at the time of re-challenge of MGA271 (irrespective of duration of first Grade 3 reaction), will permanently discontinue MGA271.
- Patients who experience a Grade 3 infusion reaction despite steroid premedication administration should permanently discontinue study drug.
- Report as an Immediately Reportable Event (IRE) within 24 hours.
- Report the event as a SAE, if appropriate.

6.4.4.4 *Grade 4:*

- Stop the infusion and disconnect the infusion tubing from the patient.
- TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING.
- Administer diphenhydramine hydrochloride 50 mg IV, methylprednisolone 2-4 mg/kg IV (or more as considered appropriate), and other medications/treatment as medically indicated (e.g., an IL-6 receptor inhibitor or IL-6 inhibitor, an IL-2 receptor inhibitor, and/or an anti-TNF $\alpha$  antibody).
- Give epinephrine or bronchodilators as indicated.
- Support ventilation and blood pressure as indicated.
- Report as an IRE within 24 hours.
- Report the event as an SAE.
- Patients who have a Grade 4 infusion reaction will not receive further MGA271.

6.4.4.5 *Grade 5:*

- Report as an IRE within 24 hours.
- Report the event as an SAE.

All changes in the infusion of MGA271 including interruption of the infusion and its duration as well as reductions in infusion rate and duration must be recorded.

#### 6.4.5 Dose Management for Adverse Events considered related to MGA271

Temporary interruptions of MGA271 may be required in the event of treatment-related toxicity. General guidelines for specific toxicity regarding dosing and treatment are provided below. All toxicities will be graded according to NCI CTCAE v4.03 (see **Section 7.1.2**).

##### 6.4.5.1 Grade 1 or 2 AEs

Study drug administration may continue despite observation of drug-related low grade adverse events (CTCAE grade 1 or 2). If the investigator in his/her medical judgment considers that a low grade AE is of clinical significance or inordinately prolonged, the Investigator may, at his/her discretion, delay treatment to allow for resolution. Necessity to delay for more than two consecutive doses will require the patient to permanently discontinue study drug. Study drug should also be discontinued for prolonged Grade 2 AEs lasting  $\geq 14$  days that are at least possibly related to study drug. No dose reductions are allowed.

##### 6.4.5.2 Grade 3 or 4 AEs

Drug administration should be held upon observation of AEs of  $\geq$  Grade 3 severity to enable patient management, monitoring of the resolution of the event, and assessment of the relatedness of the event. An AE of  $\geq$  Grade 3 that is considered related to MGA271 will result in discontinuation of therapy unless otherwise specified in the protocol. For AEs associated with liver function test abnormalities, drug administration should also be discontinued for concomitant ALT  $>3$ xULN and total bilirubin  $>2$ x ULN without an alternative etiology (Hy's Law).

## 6.5 Concomitant Therapy

MGA271 is the only cancer drug to be administered routinely in this study. No concomitant anti-cancer therapy will be given.

All concomitant medications and blood products administered during the patient's participation in the study until the post treatment follow-up visit must be recorded in the source document and on the electronic Case Report Form (eCRF). All changes in infusions, including interruptions and their duration as well as reductions in rate and duration must be recorded.

The following rules concerning concurrent treatment(s) will apply in this study:

Any other anti-neoplastic therapies including but not limited to chemotherapy or other small molecules, biologics, or radiotherapy are not allowed.

- Patients may not receive other investigational drugs during the period of study participation.
- Because MGA271 has a mechanism of action dependent upon the engagement of T lymphocytes, the use of corticosteroids should be limited to the extent possible. Chronic doses of corticosteroids in excess of 10 mg daily of prednisone or equivalent is prohibited other than for the management of drug-related adverse experiences. Steroids may be employed in the treatment of suspected MGA271-associated immune-inflammatory or autoimmune AEs in consultation with the Sponsor.
- The use of other immuno-suppressive agents is prohibited, unless they are being used to treat an adverse event.

- Use of granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor or other growth factors is prohibited.

Patients may receive the following concurrent therapy:

- Antiemetics, antidiarrheals, anticholinergics, antispasmodics, antipyretics, antihistamines, analgesics, antibiotics and other antimicrobials, histamine receptor antagonists or proton pump inhibitors, and other medications intended to treat symptoms or signs of disease.
- Transfusions such as red blood cells and platelets are permitted to treat symptoms or signs of anemia or thrombocytopenia and should be documented on the concomitant medication form.

## 6.6 Restrictions

### 6.6.1 *Prior Therapy*

Prior therapy restrictions are described in the inclusion/exclusion criteria specified in Section 3.

### 6.6.2 *Fluid and Food Intake*

There are no requirements for fasting and no restrictions for fluid and food intake by the patients during the study.

### 6.6.3 *Patient Activity Restrictions*

There are no restrictions on patient activities and no requirement for patient confinement during the study.

## 6.7 Treatment Compliance

MGA271 will be administered by healthcare professionals under the supervision of the Investigators. Records of MGA271 dose calculation, administration, and dosing regimen will be accurately maintained by site staff. The monitor will review dose calculation, administration and regimen as well as medication accountability during investigational site visits and at the completion of the study.

## 6.8 Packaging and Labeling

MGA271 will be supplied in bulk, open-label, single-use vials. All investigational product will be labelled with a minimum of the protocol number, directions for use, storage conditions, expiry date (if applicable), batch number, the statements "For clinical trial use only," and/or "CAUTION: New Drug – Limited by Federal (United States) Law to Investigational Use," and the Sponsor's name and address. Please see the Pharmacy Manual for detailed information about the packaging of the study drug.

## 6.9 Storage and Accountability

The vials containing study drug should be stored at 2°– 8° C (36°– 46° F) and must not be frozen or shaken. Protect from sunlight. To ensure compliance with storage conditions, temperature logs will be maintained.

Because there is no preservative and drug loss occurs over time, administration of study drug should begin immediately after preparation but no later than 6 hours after preparation (see Pharmacy Manual). If there is a delay in administration of study drug such that it will not be administered on the day of preparation, the Clinical Project Manager should be notified immediately. Instructions on how to proceed will be provided.

The Investigator or his/her designee is required to maintain accurate drug accountability records. A binder containing instructions and the required accountability documentation will be provided to the Investigator or his designee. When the study is completed, copies of study drug accountability records must be sent to the Sponsor. The original drug accountability records must be maintained with the rest of the documentation in accordance with Section 9.3 of the protocol.

Additional details regarding storage, handling, and accountability can be found in the Pharmacy Manual.

#### **6.10 Investigational Product Disposition at End of Study**

Upon completion or termination of the study, all unopened vials of study medication must be returned to MacroGenics or its representative, unless the site has received written authorization from MacroGenics to destroy study drug at the site. All drug returns to MacroGenics or its representative must be accompanied by the appropriate documentation and be clearly identified by protocol number and study site number on the outermost shipping container. If MacroGenics approves the destruction of drug at the site, the Investigator must ensure arrangements are made for proper disposal and that appropriate records of disposal are documented and maintained and copies provided to the Sponsor.

### **7. ADVERSE EVENTS AND REPORTING REQUIREMENTS**

An adverse event (AE) is defined as any untoward medical occurrence (symptom, sign, illness or experience) that develops or worsens in a research patient during a clinical study or within 30 days post-treatment, regardless of causality. This includes adverse clinical or laboratory findings, any adverse drug reaction (ADR), an illness with onset during the study, or an exacerbation of a preexisting illness or condition. Cancer progression should not be considered an AE, unless the investigator believes that the study treatment exacerbated the patient's condition. Exceptions are if disease progression results in death or hospitalization while a patient is on study, in which case the disease progression is considered a serious AE. Abnormal findings on physical examination or diagnostic procedures are also considered AEs if: they are associated with clinical signs or symptoms; they require therapeutic intervention or additional diagnostic testing; they lead to dose modifications/termination of the study drug; or they are considered clinically significant by the investigator. All observed or reported AEs, regardless of their suspected causal relationship to the study drug, should be recorded on the Case Report Form (CRF).

The NCI CTCAE version 4.0 will be used for adverse event descriptions and grading ([http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/ctcae\\_v3.pdf](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae_v3.pdf)). These criteria are summarized in Appendix B. The type and severity of an AE, as well as its potential link to the study drug(s), will determine whether the event requires expedited reporting in addition to routine reporting. For all AEs, the investigator must pursue and obtain information to adequately determine the causality and outcome of the event, and to assess whether it meets criteria for a serious AE. In addition, follow-up of AEs should continue until the event and any sequelae resolve or stabilize at a level acceptable to the investigator and/or the medical monitor.

#### **7.1 Recording and Grading**

##### *7.1.1 Recording*

All observed or volunteered adverse events, regardless of treatment group, severity, suspected causality, expectedness, or seriousness will be documented on the CRF.

A clinically significant change in a physical examination finding or an abnormal test result (*i.e.*, laboratory value) should be recorded as an AE, if it:

- is associated with accompanying symptoms
- requires additional diagnostic testing or medical or surgical intervention
- leads to a change in study dosing or discontinuation from the study
- requires additional concomitant drug treatment or other therapy, or
- is considered clinically significant by the investigator

An abnormal test result that is subsequently determined to be an error does not require recording as an AE even if it originally met one or more of the above criteria.

### 7.1.2 Grading severity

All adverse events will be graded for intensity on a scale of 0 to 5, according to the NCI CTCAE version 4.0 (see Table 2 and Appendix B). These criteria can be found at [http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/ctcae3.pdf](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf)

*Table 2 Intensity of Adverse Event*

<b>Grade</b>	<b>Description</b>
0 (none)	No adverse event or within normal limits.
1 (mild)	Transient or mild discomfort; generally non-progressive; no limitation in daily activities; no medical intervention required.
2 (moderate)	Mild/moderate limitation in daily activities; some assistance may be required; no/minimal medical intervention required.
3 (severe)	Marked limitation in daily activities; some assistance usually required; medical intervention is required.
4 (life-threatening)	Extreme limitation in daily activities; major assistance required; significant medical intervention required.
5 (death)	Fatal adverse event.

### 7.1.3 Attributing causality

After grading for severity, the investigator must evaluate all clinical AEs and abnormal laboratory values for possible causal relationship to the study drug(s). Causality attribution will be decided using the criteria outlined in Table 3.

*Table 3 Relationship of Adverse Event to Study Drug*

<b>Relationship</b>	<b>Description</b>
Unrelated	AE is clearly not related to the study drug (An event that does not meet any of the criteria below).
Unlikely	AE is doubtfully related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; but that could more reasonably be explained by other characteristics of the patient's clinical state).
Possible	AE may be related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or

	expected response pattern; but that could just as readily be attributed to a number of other factors).
Probable	AE is likely related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; that is confirmed by stopping/reducing the drug dose; and that could not be reasonably explained by the characteristics of the patient's clinical state).
Definite	AE is clearly related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; and that is confirmed by improvement on stopping/reducing the drug dose and reappearance on repeated exposure).

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Abnormal laboratory values of clinical significance that were present at baseline and did not change in severity or frequency during experimental therapy and those that can obviously be attributed to underlying disease will be recorded as unrelated and will not be considered when evaluating study drug toxicity.

## 7.2 Unexpected Adverse Events

An unexpected adverse event is any event not associated by nature or intensity with the investigational agent(s) under study. A comprehensive list of adverse events and potential risks related to MGA271 is provided in this Protocol and in the Consent form. The study agent may cause allergic reactions in very rare instances. A severe allergic reaction could be life-threatening. Examples of allergic reactions include: rash; shortness of breath; wheezing; sudden drop in blood pressure; swelling around the mouth, throat, or eyes; fast pulse; and sweating.

## 7.3 Serious Adverse Events and Serious Adverse Drug Reactions

The investigator must assess each event to determine if it meets the criteria for classification as a serious adverse event (SAE) or serious adverse drug reaction (ADR). An SAE/ADR is defined in the Code of Federal Regulations (21CFR312.32) as any event that:

- results in death
- is life-threatening
- results in inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- results in congenital anomaly or birth defect
- is medically significant in the opinion of the investigator

All SAEs that occur any time while a patient is on study (*i.e.*, as soon as the informed consent has been signed) or within 30 days of the last dose of study drug administration must be documented, regardless of the suspected relationship to the investigational agent(s). Any SAE occurring more than 30 days after the last dose of the study drug(s) must be recorded if a causal relationship to the investigational agent(s) is suspected.

### 7.3.1 Progression of malignancy

Progression of a patient's malignancy should *not* be considered an AE unless, in the investigator's opinion, study treatment resulted in an exacerbation of the patient's condition.

If disease progression results in death or hospitalization while on study or within 30 days of the last dose of study drug administration, progressive disease will be considered an SAE.

#### 7.3.2 *Life-threatening events*

A life-threatening event is any AE that places the patient at immediate risk of death from the reaction as it occurs. It is not a reaction that, had it occurred in a more severe form, might have caused death.

#### 7.3.3 *Hospitalization or prolongation of hospitalization*

Hospitalization encompasses any inpatient admission (even if < 24 hours) resulting from a precipitating treatment-emergent adverse event. For chronic or long-term patients, inpatient admission also includes transfer within the hospital to an acute or intensive care inpatient unit. Hospitalizations for administrative reasons or a non-worsening preexisting condition should not be considered AEs (*e.g.*, admission for workup of a persistent pretreatment laboratory abnormality, yearly physical exam, protocol-specified admission, or prostatectomy surgery). Hospitalization because of an unplanned event will be deemed an SAE. Preplanned treatments or surgical procedures should be noted in the baseline documentation. In the case of this study, all patients will have a preplanned prostatectomy surgery.

Prolongation of hospitalization is any extension of an inpatient hospitalization beyond the stay anticipated or required for the original reason for admission.

#### 7.3.4 *Significant disability*

This is defined as a substantial disruption of the patient's ability to conduct normal life functions and activities of daily living.

#### 7.3.5 *Congenital anomaly*

If the female partner of a male patient becomes pregnant during the course of the study, the treating physician must be notified immediately. All confirmed pregnancies must be immediately reported to the principal investigator and the medical monitor, and recorded in the CRF. All pregnancies will be followed until resolution (*i.e.*, voluntary or spontaneous termination or birth) and assessed for congenital anomalies and birth defects.

#### 7.3.6 *Medical significance*

An event that is not fatal or life-threatening and that does not necessitate hospitalization may be considered serious if, in the opinion of the investigator, it jeopardizes the patient's status and might lead to medical or surgical intervention to prevent any of the above outcomes. Such medically significant events could include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

### 7.4 **Perioperative Adverse Events**

Complications of surgery or those occurring in the early post-operative period will be recorded. These include wound complications, estimated blood loss, post-operative infections, delayed wound healing, abnormal laboratory values, etc. Duration of hospital stay will be recorded. Additional medical examinations will be allowed at the request of patients during drug administration or at the discretion of the principal investigator for the evaluation of new adverse events that warrant physical examination. During and following completion of the study, patients should notify the study

staff of any problems that occur between visits or following study termination by telephone and, if necessary, will be evaluated by the investigator or study personnel at an unscheduled interim visit.

Operative/perioperative events will be recorded as described in Section 7.1, and their severities will be categorized using the NCI CTCAE version 4.0 criteria. The relationship of these events to the investigational drug(s) will be determined by the principal investigator together with urologist co-investigators and, if necessary, with the primary urologic surgeon. Reporting of these events will follow the same guidelines described in Section 7.5.

## 7.5 Reporting Adverse Events

### 7.5.1 Reporting serious adverse events (SAEs)

IND application sponsors are required to notify FDA in a written safety report of:

- any adverse experience associated with the use of the drug that is both serious and unexpected or
- any findings from tests in laboratory animals that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, and carcinogenicity.

**Adverse event** means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

**Suspected adverse reaction** means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

**Adverse reaction** means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

**Unexpected** adverse event or suspected adverse reaction refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed; or, if an investigator's brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current IND application.

**Serious** adverse event or suspected adverse reaction refers to an event or reaction that, in the view of either the investigator or sponsor, results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- in-patient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly or birth defect.

**Life-threatening** adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize

the patient or research subject and may require medical or surgical intervention to prevent one of the outcomes listed as serious.

**Mandatory Safety Reporting**

- **Initial reporting:** *IND application sponsor must report any suspected adverse reaction or adverse reaction to study treatment that is both serious and unexpected.*

*Unexpected serious suspected adverse reactions suggesting significant risk to human subjects must be reported to FDA as soon as possible but no later than within **15 calendar days** following the sponsor's initial receipt of the information.*

*Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and must be reported to FDA as soon as possible but no later than **7 calendar days** following the sponsor's initial receipt of the information.*

- **Follow-up reporting:** *Any relevant additional information obtained by the sponsor that pertains to a previously submitted IND safety report must be submitted as a Follow-up IND Safety Report. Such report should be submitted without delay, as soon as the information is available but no later than 15 calendar days after the sponsor receives the information.*

*All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The type of report (initial or follow-up) should be checked in the respective boxes on Forms 3500A and 1571.*

*The submission must be identified as:*

- *"IND safety report" for 15-day reports, or*
- *"7-day IND safety report" for unexpected fatal or life-threatening suspected adverse reaction reports, or*
- *"Follow-up IND safety report" for follow-up information.*

*The report must be submitted to an appropriate Review division that has the responsibility to review the IND application under which the safety report is submitted. Each submission to this IND must be provided in triplicate (original plus two copies). Send all submissions to the following address:*

*Food and Drug Administration  
Center for Drug Evaluation and Research  
Division of Oncology Products 1  
5901-B Ammendale Road  
Beltsville, MD 20705-1266*

If the IND is not in eCTD (Electronic Common Technical Document format), other means of rapid communication (e.g., telephone, facsimile transmission, email) may be used. If the IND is not in eCTD format and the sponsor intends to submit 7-day IND safety reports by facsimile transmission or email, the sponsor should address the submissions to the Regulatory Project Manager and the Chief, Project Management Staff in the FDA review division that has responsibility for review of the IND.

**7.5.2 Reporting requirements for the Sidney Kimmel Comprehensive Cancer Center (SKCCC)**

The principal investigator will notify the appropriate regulatory agencies of any SAEs occurring during the study period, regardless of causality. These agencies include the Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data and Safety Monitoring Committee (DSMC), and the Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC) of the Johns Hopkins Medical Institutions (JHMI). Expedited reporting to the IND Sponsor by the PI within 24 hours is required for all SAEs (see Section 7.4.1). For SAEs that

are fatal, life-threatening, or treatment-related but non-life threatening; IRB/IBC reporting by the PI is required within 3 days. For unrelated SAEs, IRB/IBC reporting by the PI is required within 15 days. All other AEs should be documented on CRFs and submitted according to the standard data management guidelines.

Adverse event information will be collected continuously throughout the duration of the study. Participants will be instructed to notify their treating provider of any new signs or symptoms, and providers will actively assess patients for adverse events at each visit (including by evaluation of laboratory studies). The investigator will assess each AE for its severity and for its relationship to the study drug, and all events Grade 1 or higher will be documented on CRFs and then reported as described above within the required time frame. Any AE occurring while a patient is on study (*i.e.* after informed consent has been signed) or within 30 days of study termination requires reporting. AEs occurring later than this must still be reported if a causal relationship with the study drug is suspected.

For all AEs, the investigator must pursue and obtain information to adequately determine the causality and outcome of the event, and to assess whether it meets criteria for a SAE. In addition, follow-up of an AE is required until the event either resolves or stabilizes. Initial reporting of an AE should include at a minimum the patient number, age, the dose at which the event occurred, and the type and severity of the event. Follow-up information including causality, duration, outcome, action taken, and concomitant medications should be reported soon thereafter.

The principal investigator must keep copies of all CRFs and other AE information, including correspondence with the IRB and/or FDA, for as long as required to comply with national and international regulations (generally at least 3 years after study completion).

## 7.6 Pregnancy

Pregnancy is not an AE unless it results in congenital anomaly or birth defect, in which case it is a SAE. If the partner of a male patient should become pregnant while he is participating in the study, the patient should inform his treating physician immediately. All pregnancies must be reported at once to the principal investigator and medical monitor. All confirmed pregnancies will be followed until birth or until voluntary or spontaneous termination.

## 8. OUTCOME ASSESSMENT

### 8.1 Radical Prostatectomy Specimen

Most of the study outcomes in this immunologic trial will depend on collection of prostate tissue from prostatectomy specimens. All pathologic specimens will be handled in routine fashion by the operating room (OR) staff, except that as soon as the specimen is removed the OR nurse will directly page a tissue harvesting technician that is part of the Brady Urological Research Institute Prostate Specimen Repository Team. Accessioning of pathology specimens will be coordinated in the OR areas by the Departments of Urology and Pathology. A study technician will be available to the pathologist receiving the specimen to assure that the tissue is handled appropriately for the intended bioassays. At the time of harvesting, the pathologist will apply ink and cut the prostate specimen in transverse sections. The specimen will be fixed in 10% phosphate buffered formalin, and tissue blocks will be paraffin-embedded and sectioned at 4  $\mu$ m thickness for routine histologic evaluation and for immunohistochemical determinations. Fixation should occur as soon as possible after operative removal of the prostate, and ideally within 30-60 minutes. Assessment of index tumors for Gleason grade, nodal involvement, and pathologic staging will be conducted in usual fashion and will be provided to the patient for prognostic information. After the pathology report is available, a database

will be established and all information from the pathology reports of all samples will be included. Tumor blocks and/or additional unstained slides will be collected for study-specific quantitative immunohistochemical evaluations.

## 8.2 Primary Endpoint

### 8.2.1 Rate of adverse events

All subjects receiving at least one dose of the study drug will be evaluated for safety by monitoring symptoms, physical examinations, and laboratory tests. Adverse events will be classified and graded according to the NCI Common Toxicity Criteria version 4.0 (see Appendix B). The absolute number and frequency of each adverse event will be reported, and subdivided according to toxicity grade. A description of adverse events by treatment arm will also be reported. A particular adverse event occurring more than once in the same subject will be counted only once and at its worse grade.

### 8.2.2 In expansion cohort (amendment 1):

- All subjects receiving at least one dose of the study drug will be assessed for PSA<sub>0</sub> Response Rate (Undetectable PSA level <0.1 ng/mL) at 12 months following radical prostatectomy

## 8.3 Secondary and Exploratory Endpoints

### 8.3.1 Markers of apoptosis

The primary measure of treatment effect in this study will be achieved by quantification of tumor cell apoptosis in prostate tumor specimens of treated patients. Tissue microarrays (TMAs) from formalin-fixed tumor samples will be analyzed for the degree of tumor apoptosis using immunohistochemical staining for activated caspase 3 (Marcelli et al 2000), or by using the method of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (*i.e.* TUNEL assay) (Furuya et al 1995). The *in situ* Cell Death Detection Kit (Roche, Indianapolis, IN) may be used to perform TUNEL reactions. Quantification of staining percentage will be achieved using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue. Analyses will be performed in the laboratory of Dr Angelo DeMarzo. The contact information for the DeMarzo laboratory is listed below:

Angelo DeMarzo, MD, PhD  
CRB1 - Room 151  
1650 Orleans Street  
Baltimore, MD 21231  
Phone: (410) 614-5686  
Fax: (410) 502-9817  
Email: [ademarz@jhmi.edu](mailto:ademarz@jhmi.edu)

### 8.3.2 Markers of cell proliferation

TMAs from fixed tumor samples will be analyzed for the degree of tumor cell proliferation using the validated marker, Ki-67. This will be achieved by immunohistochemical staining (Berges et al 1995; Rubin et al 2002), using the Ki-67 monoclonal antibody (Dako North America, Carpinteria, CA). Quantification of staining percentage will be performed using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) as described in Section

8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue. Assays will be performed in the laboratory of Dr DeMarzo.

#### 8.3.3 *CD8<sup>+</sup> T cell infiltration*

To assess the immune response to MGA271, we will quantify the extent of CD8<sup>+</sup> T cell infiltration into the prostate from harvested prostate glands. This will be done using immunohistochemical staining methods. This endpoint will be expressed as the mean CD8<sup>+</sup> T cell staining percentage in harvested tumor tissue. We will also attempt to quantify prostatic CD4<sup>+</sup> T cell infiltration and T<sub>reg</sub> infiltration, as well as to determine the CD8/T<sub>reg</sub> ratio and the CD4/T<sub>reg</sub> ratio.

Analysis of this endpoint will be achieved by preparing tissue microarrays (TMAs) using the highest-grade/largest tumor per patient and sampling it with 100-fold redundancy. All tissues will first be fixed in 10% neutral buffered formalin and processed into paraffin blocks. For each immunohistochemical stain (*e.g.* CD8<sup>+</sup> T cell stain, CD4<sup>+</sup> T cell stain, T<sub>reg</sub> stain), TMA slides will be scanned using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) and TMA cores will first be assigned a diagnosis by the study pathologist and will then be subjected to semi-automated image analysis using the Aperio system. For each biomarker, we will divide the area of brown DAB staining by the area of epithelial cells on the TMA core, obtaining a staining percentage. The area of epithelium will be obtained on each TMA core by staining with cytokeratin-8 and using automated image analysis. Cores with both tumor and normal tissue will be excluded if they contained >10% of the other component.

For CD8 staining, slides will be steamed for 20 minutes in citrate antigen retrieval solution (Vector Laboratories, Burlingame, CA) followed by incubation with a mouse monoclonal anti-CD8 antibody for 45 minutes at room temperature (Dako, Carpinteria, CA). For CD4 staining, slides will be steamed for 40 minutes in high pH antigen retrieval solution (Dako, Carpinteria, CA) and then incubated with a mouse monoclonal anti-CD4 antibody for 45 minutes at room temperature (Serotec, Kidlington, UK). For T<sub>reg</sub> analysis, cells will be stained for the FoxP3 protein by steaming slides for 40 minutes in high pH antigen retrieval solution (Dako, Carpinteria, CA) and incubating them with a mouse monoclonal anti-FoxP3 antibody for 45 minutes at room temperature (eBioscience, San Diego, CA, 1:1000 dilution). In all cases, poly-HRP-conjugated anti-mouse IgG Ab (Dako, Carpinteria, CA) will be used as the secondary antibody. Staining will be visualized with diaminobenzidine (Sigma, Saint Louis, MO) and slides will be counterstained with hematoxylin.

Images of each TMA core will be captured by automated scanning of TMA slides using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA). Captured images will be imported into the TMAJ Images Application program (<http://tmaj.pathology.jhmi.edu>). Histological diagnoses (normal, atrophy, prostatic intraepithelial neoplasia, cancer) will be applied to all images used for the analyses by a pathologist. In addition, for TMA spots containing more than one type of lesion, the percentage of each diagnosis will be noted. All images and data will be available for viewing/downloading at <http://demarzolab.pathology.jhmi.edu/Pubs.html>. For image analysis, we will use a custom open source software package, FRIDA (Framework for Image Dataset Analysis; <http://sourceforge.net/projects/fridajhu>), for the evaluation of red-green-blue (RGB) color image datasets, including those generated from scanning of tissue microarray slides. To analyze CD4, CD8 and FoxP3 (T<sub>reg</sub>) staining, hue-saturation-brightness (HSB) segmentation ranges for brown DAB staining will be defined from the tissue microarray image set, and the total number of pixels in every image that fall within the defined parameters for brown DAB staining will be calculated, reflecting the total area of brown DAB staining for each spot. For

every spot, a “staining ratio” for each of the three proteins will be calculated by dividing the total area (in pixels) of brown DAB staining by the average TMA spot area.

Previous TMA studies conducted by Angelo DeMarzo, MD PhD and Charles G. Drake, MD PhD have defined the extent of T cell infiltration into human prostate glands using prostatectomy specimens. In normal prostate tissue, the mean staining percentage for CD8<sup>+</sup> T cells is 0.29% (interquartile range, IQR, 0.13% - 0.39%) while in tumor tissue, the mean percentage of CD8<sup>+</sup> T cells is 0.42% (IQR 0.07% - 0.71%). The corresponding values for CD4<sup>+</sup> T cell infiltration in normal and tumor tissue are 0.16% (IQR 0.04% - 0.18%) and 0.25% (IQR 0.01% - 0.32%), respectively. The corresponding values for T<sub>reg</sub> infiltration in normal and tumor tissue are 0.03% (IQR 0.02% - 0.04%) and 0.06% (IQR 0.02% - 0.08%), respectively (Gurel and DeMarzo, unpublished data).

The above analyses will be performed in the laboratory of Angelo DeMarzo, MD PhD. A detailed description of these methodologies has previously been published (Zha et al 2001; Faith et al 2004; Gurel et al 2008).

#### 8.3.4 *PD-L1 expression*

PD-L1 expression in prostate tumor specimens will be assessed by IHC in the primary core specimens (pre-treatment) and in the prostatectomy surgical specimens (post-treatment). This endpoint will be expressed as the mean staining percentage of PD-L1 in tumor tissue.

#### 8.3.5 *Regulatory T cell (T<sub>reg</sub>) infiltration*

The method for quantifying T<sub>reg</sub> density from harvested prostate tissue is as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue.

#### 8.3.6 *CD4<sup>+</sup> T cell infiltration*

The method for quantifying CD4<sup>+</sup> T cell density from harvested prostate tissue is as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue.

#### 8.3.7 *Mean NK cell density in tumor tissue from harvested prostate glands of patients.*

The method for quantifying NK cell density from harvested prostate tissue is as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue.

#### 8.3.8 *FC Receptor Genotyping*

Determination of Fc receptor genotype (CD16A, CD32A, CD32B). To be shipped to MacroGenics for analysis.

#### 8.3.9 *Sera for Immunoassays*

*Sera for immunoassays will be collected at each time point (Pre-treatment, Radical Prostatectomy, and Follow-up),*

#### 8.3.10 *PBLs*

*Whole blood (100cc) will be collected for PBLs at each time point ((Treatment Day 1, Treatment Day 36, and Follow-up day 30),*

#### 8.3.11 *TCR Repertoire*

To assess changes in TCR repertoire in peripheral and tumor T-cells following treatment with MGA271, TCR Deep Sequencing analysis will be performed via Adaptive Biotechnology (Seattle, WA). For tumor T-cell analysis, a tumor sample (10 mg fresh frozen or 25 mg FFPE) will be used. For peripheral T-cell analysis, peripheral whole blood samples (2x10 mL) pre-treatment, at time of surgery, and 30 and 90 days post-op will be used. Note: Adaptive Biotechnology requires a min of 3ug DNA (tissue or blood) for deep sequencing.

#### 8.3.12 *B7-H3 expression*

B7-H3 expression in prostate tumor specimens will be assessed by IHC in the primary core specimens (pre-treatment) and in the prostatectomy surgical specimens (post-treatment). This endpoint will be expressed as the mean staining percentage of B7-H3 in tumor tissue.

#### 8.3.13 *Enoblituzumab (MGA271) drug distribution*

To analyze MGA271 drug levels in prostate tumor specimens of treated patients, fresh frozen sections will be evaluated by IHC for evidence of MGA271 drug distribution. Fresh frozen section samples will be shipped to MacroGenics for analysis. This endpoint will be expressed as positive or negative detection of MGA271 in tumor tissue.

#### 8.3.14 *Tissue androgen concentrations*

Tissue concentrations of testosterone and 5 $\alpha$ -dihydrotestosterone (DHT) will be measured using a highly sensitive liquid chromatography–electrospray ionization tandem mass spectrometry method using a high proton affinity derivatization of the 17 $\beta$ -hydroxyl group of testosterone and DHT with picolinic acid, and a mobile phase consisting of MeCN–MeOH–H<sub>2</sub>O–formic acid and a conventional octadecylsilica (ODS) column (Yamashita et al 2009). Purification of the derivatives will be carried out using solid-phase extraction with the ODS cartridge. By this method, testosterone and DHT will be determined simultaneously with limits of quantification of 0.5 pg and 1 pg/3 mg of prostate tissue, respectively.

#### 8.3.15 *Androgen receptor (AR) quantification*

The method for quantifying androgen receptor (AR) density from harvested prostate tissue is similar to that described in Section 8.3.3, and will rely on immunohistochemical staining for the AR protein. This endpoint will be expressed as the mean staining percentage in tumor tissue.

#### 8.3.16 *Pathological complete responses (pCR)*

This will be defined as the absence of tumor identification by the study pathologist on standard histological analysis of the resected prostate specimens. The endpoint will be expressed as the proportion of men achieving a pCR.

#### 8.3.17 *PSA response rates*

This will be defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 3 months after prostatectomy. The endpoint will be expressed as the proportion of men achieving a PSA response.

#### 8.3.18 *Time to PSA recurrence*

This will be defined as the interval from the time of prostatectomy to the time when the serum PSA is  $\geq$ 0.2 ng/mL. PSA will be measured approximately 1 month after prostatectomy, and every 3 ( $\pm$ 1) months during the first post-operative year and every 6 ( $\pm$ 2) months during the second and third post-operative years. For subjects who have not yet demonstrated PSA relapse at the time of censoring, patients will be censored at the date of

the last assessment that shows a lack of PSA recurrence. This outcome will be expressed as a median and will be determined using the Kaplan-Meier method.

*8.3.19 In expansion cohort (Amendment 1): assessment of Gleason grade change (prostatectomy Gleason sum vs. core biopsy Gleason sum) post neoadjuvant Enoblituzumab treatment*

This will be defined by comparing highest Gleason grade from pre-treatment biopsy versus highest Gleason grade from post-treatment prostatectomy. This endpoint will be expressed as the proportion of men achieving a Gleason score change.

*8.3.20 Global expression profiling of pre and post treatment tumor tissue using single cell RNA sequencing, the immune NanoString immunopanel and/or microarrays*

Expression profiling will be conducted as per established protocols for single cell RNA seq, Nanostring, and microarray

*8.3.21 IHC analyses of CD137, CD16 and/or CD107A across potential immune infiltrate following Enoblituzumab*

CD137, CD107A, and CD16 expression in prostate tumor specimens will be assessed by IHC in the prostatectomy surgical specimens (post-treatment). This endpoint will be expressed as the mean staining percentage of each of these in tumor tissue.

## **9. REGULATORY AND REPORTING REQUIREMENTS**

Contact details for personnel connected with this study are provided on the title page at the front of this protocol. Patient registration procedures are described in Section 4.

### **9.1 Regulatory Responsibilities**

*9.1.1 Protocol chair*

The protocol chair (=PI) is responsible for the following tasks:

- Coordinating, developing, writing, submitting, and obtaining IRB-approval for the protocol as well as its subsequent amendments.
- Assuring that all study personnel are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study and for monitoring the progress of the study.
- Reviewing and ensuring reporting of serious adverse events (SAEs).
- Reviewing data from all patients.

*9.1.2 Study Coordinator*

The study coordinator is responsible for the following tasks:

- Ensuring that IRB approval has been obtained prior to patient registration, and maintaining copies of IRB approvals (including approval of amendments).
- Managing patient registration.
- Collecting and compiling data from each patient.

- Establishing procedures for documentation, reporting, and submission of AEs/ SAEs to the protocol chair (Name) and other applicable parties.
- Facilitating audits by securing selected source documents and research records from participating patients for audit.

### 9.1.3 *Study personnel*

Study personnel (co-investigators, research nurses) are responsible for these tasks:

- Following the protocol as written, and Good Clinical Practice (GCP) guidelines.
- Submitting data to the project manager.
- Registering all patients by submitting the patient registration form and signed informed consent form promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct the trial according to the protocol.
- Maintaining regulatory binders and providing copies of all required documents to the project manager.
- Collecting/submitting data according to the schedule specified by the protocol.

## 9.2 **Data Management**

Data collected during this study will be entered into a secure database. The study coordinator will be responsible for the initial study configuration and setup of the database and for any future changes.

### 9.2.1 *Case report forms*

Case report forms (CRFs) will be generated by the study coordinator for the collection of all study-related data. Investigators and study personnel will be responsible for ensuring that the CRFs are kept up-to-date.

### 9.2.2 *Source documents*

Investigators and study personnel will record clinical data in each patient's source documents (*i.e.*, the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with the source documents.

### 9.2.3 *Record retention*

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator should maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 3 years after the completion of all study-related activities.

## 9.3 **Study Monitoring and Quality Assurance**

This is a DSMP Level II study under the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data Safety Monitoring Plan (12/6/2012) Data Monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by the Principal Investigator and

externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

Additionally, scheduled meetings will take place monthly and will include the protocol principal investigator, research nurse, data manager, and, when appropriate, the collaborators, subinvestigators, and biostatistician involved with the conduct of the protocol.

During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

## 10. STATISTICAL CONSIDERATIONS

### 10.1 Study Design and Sample Size

This is a single-center, single arm, open-label phase II study evaluating the safety, anti-tumor effect, and immunogenicity of neoadjuvant MGA271 given weekly for 6 doses beginning 50 days prior to radical prostatectomy in men with high-risk localized prostate cancer. Our primary objective is to characterize safety, tolerability, and feasibility of treatment of men with Enoblituzumab in the neoadjuvant setting. The trial will monitor toxicity and safety, as well as surgery related adverse events. (see Section 10.2.1). The secondary objective will be to evaluate immune response consistent with the agent's proposed mechanism of action (MOA), antibody-dependent cellular cytotoxicity (ADCC). Tumor cell death will be quantified by Caspase 3 staining, and post-treatment apoptotic index compared with that from the pre-treatment biopsy. The sample size is driven by detecting a pharmacodynamic effect of the agent based on the key secondary endpoint of tumor cell death. A biologically meaningful treatment effect will be deemed to be one where the tumor cell apoptosis is two-fold higher after MGA271 therapy. Previous study showed that baseline apoptosis, measured by Caspase staining, is  $0.06 \pm 0.08$  (Mean  $\pm$  SD) for Gleason 4 and  $0.2 \pm 0.2$  for Gleason 3 (Kim et al. 2016). Based on this data, we assume that the coefficient of variation of tumor cell death measure is 1.2 and the correlations between the measures pre- and post-treatment is moderate at 0.4, 16 subjects will provide 88% power to detect a 2-fold increase in apoptotic tumor cells after the treatment compared to baseline, using a one-sided paired t-test with significance level 0.05.

In Amendment 1, the study was expanded to enroll an additional 16 patients for a total of 32 patients to continue evaluating safety and better estimate the clinical benefit of Enoblituzumab in terms of undetectable PSA level ( $<0.1$  ng/mL) at 12 months following radical prostatectomy.

Patients will be replaced if they are removed from the study after signing the informed consent but before undergoing radical prostatectomy. Patients receiving at least one dose of the study drug will be included in both safety and efficacy analyses.

### 10.2 Study Endpoints

#### 10.2.1 Analysis of the primary endpoints

##### Safety and feasibility

Frequency of adverse events will be described using summary statistics. The proportion of patients with an adverse event will be reported with an exact binomial 95% confidence interval. All subjects receiving at least one dose of the study drug(s) will be evaluable for toxicity.

**Early stopping rules for toxicity:** Toxicity will be monitored closely and continuously. Any grade-3/4 local reactions (erythema, swelling, pain) attributable to MGA271 or Grade 3 infusion-related reaction or cytokine release syndrome that lasts  $\geq$  12 hours will be considered AEs for purposes of this stopping rule. A grade 4 infusion related reaction of any duration will be considered AEs for purposes of this stopping rule. If the risk of these adverse events appears to be higher than 33%, we will temporarily halt the study pending dose modification. Specifically, we apply a Bayesian toxicity monitoring rule that suspends the enrollment if the posterior probability of risk being larger than 0.33 is 65% or higher. The monitoring rule uses beta (1.5, 5.5) as prior distribution. This means that our prior guess at the proportion of toxicity is 21%, and there is 90% probability that this proportion is between 3% and 49%. The decision rule for toxicity stopping is as follows:

Study termination if:	3 AEs	4 AEs	5	6	7	8	9	10	11	12	13
In number of patients between:	3 - 4	5 - 7	8 - 10	11 - 12	13 - 15	16 - 18	19 - 21	22 - 24	25 - 27	28 - 30	31 - 32

For example, we will interrupt the accrual if 3 patients have AEs among the first 4 receiving treatment. If 4 or more out of the first 5-7 patients have AEs, we will suspend accrual.

The operating characteristics of the stopping rule are shown below and are based on 5000 simulations:

Risk of AE	0.20	0.25	0.30	0.33	0.40	0.45
% of Time study stops	7.5%	18%	33.7%	46%	54.6%	73.3%
Expected sample size	30.3	28.2	25.3	22.8	21.2	17.3

**Early stopping rule for feasibility:** We do not anticipate an increase in the surgical difficulty with the use of neoadjuvant MGA271. Men who have had acute (post-biopsy) and prolonged issues (chronic prostatitis) with prostate infection/inflammation are fairly routine in urologic surgical practice, and we anticipate that the immune infiltrate potentially induced by MGA271 would not pose a substantial increase in surgical difficulties. However, in addition to safety, feasibility will be monitored separately. The feasibility rule for this study will be based on a change in surgical outcomes beyond what may be expected for patients without presurgical interventions and that may be attributable to the study drug. These events would include: (1) average blood loss in excess of 2500 mL, (2) average operative time in excess of 3.5 hours, and (3) average hospital stay in excess of 4 days, (4) systemic symptoms (fevers, rash, myelosuppression) compromising the planned surgery. These values are approximately 2 standard deviations above the average surgical outcomes for men undergoing radical prostatectomy at Johns Hopkins Hospital.

We expect no surgery complication in at least 90% cases. We will monitor this endpoint after every patient. If it becomes apparent that the surgeries are being negatively impacted by the pre-surgical investigational treatments, then the study will be suspended for review. Again, we apply a Bayesian monitoring rule that suspends the enrollment if the posterior probability of complications  $> 0.10$  is 80% or higher. The monitoring rule uses beta (1, 9) as prior distribution, based on the expectation that these treatments will not impact the

feasibility of surgery. Instances where the study would be temporarily suspended are listed below.

Stopping rules:

Not feasible if the observed number of surgical complications is:	2	3	4	5	6
In number of patients between:	2 - 6	7 - 14	15 - 22	23 - 30	31 - 32

The operating characteristics of the stopping rule are shown below and are based on 5000 simulations:

Risk of surgical complication	0.025	0.05	0.10	0.15	0.20
% of Time study stops	1.1%	5.9%	29.2%	57.7%	81.4%
Expected sample size	31.7	30.7	26.4	20.9	15.3

*10.2.1.1 For expansion Amendment 1*

Estimation of PSA<sub>0</sub> Response Rate (Undetectable PSA level <0.1 ng/mL) at 12 months following radical prostatectomy

Our primary efficacy endpoint will be to estimate the clinical benefit of neoadjuvant Enoblituzumab in terms of undetectable PSA level (<0.1 ng/mL) at 12 months following radical prostatectomy. The estimate and corresponding 90% confidence interval based on 32 patients is listed in the table below.

Observed N with undetectable PSA	Observed rate	90% CI
0	0	0% - 7.8%
1	3.1%	0.7% - 12.9%
2	6.2%	2.1% - 17.2%
3	9.4%	3.8% - 21.3%

*10.2.2 Analysis of the secondary and exploratory endpoints*

The secondary endpoints of this study have previously been defined (see Section 8.3). The statistical analysis of these endpoints is described below. Data transformation will be performed when they are not normally distributed.

- **Apoptotic markers.** The primary objective of pharmacodynamics effect of this study is to determine the immunologic response consistent with the agent's proposed mechanism of action (MOA), antibody-dependent cellular cytotoxicity (ADCC). Tumor cell death will be quantified by TUNEL staining, Caspase 3 staining, and FcGamma staining and will be expressed as the mean staining percentage in tumor samples. Standard deviations, 95% confidence intervals, and ranges will also be reported where appropriate. Means will be

compared pre-treatment (from pre-treatment biopsies) vs. post-treatment using paired sample test.

- **Proliferation markers.** Ki-67 staining will be expressed as the mean staining percentage in tumor samples. Standard deviations, 95% confidence intervals, and ranges will also be reported where appropriate. Means will be compared pre-treatment vs. post-treatment using paired sample test.
- **CD8<sup>+</sup> T cell infiltration.** Mean CD8<sup>+</sup> T cell staining percentage in harvested prostate tissues will be reported. The standard deviation, 95% confidence interval, median, and range of values will also be reported where appropriate. Since the CD8<sup>+</sup> T cell quantity is a ratio variable and the distribution is skewed, the log transformation will be used for the analysis. Tissue microarrays (TMAs) using the highest-grade/largest tumor per patient and sampling it with 100-fold redundancy will be used for CD8<sup>+</sup> T cell quantification. We expect that 3-50% of the spots per patient will be assigned a carcinoma diagnosis by the study pathologist. Cores with both tumor and normal tissue will be excluded if they contain >10% of the other component. The percent positive staining score for CD8<sup>+</sup> T cells in the spots classified as tumor will be used to quantify the outcome. The mean will be used to pool multiple spot measurements for each patient.
- **PD-L1+ cell density.** The methods for quantifying and analyzing PD-L1 cell density will be similar to those described for CD8<sup>+</sup> T cell infiltration. Descriptive statistics and graphical summaries will be provided.
- **Regulatory T cell (T<sub>reg</sub>) density.** The methods for analyzing T<sub>reg</sub> infiltration will be similar to those described for CD8<sup>+</sup> T cell infiltration. Descriptive statistics and graphical summaries will be provided. In addition, the CD8<sup>+</sup>/T<sub>reg</sub> ratio and the CD4<sup>+</sup>/T<sub>reg</sub> ratio will be computed, and reported using descriptive statistics.
- **CD4<sup>+</sup> T cell density.** The methods for quantifying and analyzing CD4<sup>+</sup> T cell infiltration will be similar to those described for CD8<sup>+</sup> T cell infiltration. Descriptive statistics and graphical summaries will be provided.
- **Mean NK cell density.** The methods for quantifying and analyzing NK cell infiltration will be similar to those described for CD8<sup>+</sup> T cell infiltration. Descriptive statistics and graphical summaries will be provided.
- **Tissue androgen concentrations.** Intraprostatic androgen concentrations (*i.e.* testosterone and dihydrotestosterone) will be summarized descriptively.
- **Enoblituzumab (MGA271) drug distribution.** Intraprostatic Enoblituzumab staining concentrations will be summarized descriptively.
- **Androgen receptor (AR) quantification.** Androgen receptor (AR) staining concentrations will be summarized descriptively.
- **Pathological complete responses (pCR).** This will be defined as an absence of tumor identification on standard histological analysis of the resected prostate specimens.
- **PSA response rates.** This will be defined as an undetectable PSA (<0.1 ng/mL) at 3 months after prostatectomy. The proportion of patients achieving a PSA response will be reported. .
- **Time to PSA recurrence.** This will be defined as the interval from prostatectomy to the time when the serum PSA is ≥0.2 ng/mL. For subjects who have not yet demonstrated PSA recurrence at the time of censoring, patients will be censored at the date of the last

assessment that shows a lack of PSA recurrence. The median time to PSA recurrence after prostatectomy (*i.e.*, the median PSA-recurrence-free survival) will be estimated with 95% confidence intervals using Kaplan-Meier survival analysis.

- **Gleason grade change** post neoadjuvant Enoblituzumab treatment from pre-treatment biopsy. This will be calculated utilizing the highest Gleason grade from the pre-treatment biopsy and the post-treatment prostatectomy.

### 10.3 Analysis Populations

#### 10.3.1 *Intention-to-treat population*

All patients who meet eligibility criteria and receive at least one dose of the study drug will be included in the analysis of the primary and secondary endpoints, even if there are subsequent protocol deviations. However, in cases where prostatectomy is not performed or if adequate surgical tissue is not collected, then determination of the secondary endpoints (apoptosis/proliferation marker analysis, tissue CD8<sup>+</sup> T cell analysis) will not be possible. Patients will be replaced if they are removed from the study after signing the informed consent but before receiving the study drug.

#### 10.3.2 *Safety population*

All patients enrolled in the study will be included in the safety analysis population and considered evaluable for toxicity from the time of their first dose of the study drug(s). Patients never receiving any of the study drugs will not be included in this analysis. Demographic and baseline characteristics for the safety population will be summarized by number and percent for categorical data and by descriptive statistics for continuous data.

### 10.4 Safety Analysis

#### 10.4.1 *Evaluation of adverse events*

Treatment-emergent adverse events will be translated from investigator terms to MedDRA version 6.0 terminology and summarized (number and percentage of patients) for all patients who receive at least one dose of the study drug(s). Adverse event summaries will be organized by body system, frequency of occurrence, intensity (*i.e.*, severity grade), and causality or attribution. Patients who experience an adverse event more than once will be counted only once. The occurrence with the maximum severity will be used to calculate intensity.

#### 10.4.2 *Evaluation of serious adverse events and premature withdrawals*

Adverse events deemed serious and those resulting in early treatment withdrawal or death will be summarized separately. Narrative paragraphs will be generated to describe the circumstances surrounding each SAE and each death.

#### 10.4.3 *Evaluation of laboratory parameters and assays*

Abnormal laboratory parameters (*e.g.* electrolyte levels, liver function tests, renal function tests, complete blood counts) will be summarized, and clinically significant changes from baseline will be discussed.

## **11. PROTECTION OF HUMAN SUBJECTS**

### **11.1 Ethical Considerations**

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) guidelines established by the International Conference on Harmonization (ICH), and the ethical standards set forth in the Declaration of Helsinki of 2004 (these documents may be found at [www.wma.net/e/policy/b3.htm](http://www.wma.net/e/policy/b3.htm) and [www.laakariliitto.fi/e/ethics/helsinki.html](http://www.laakariliitto.fi/e/ethics/helsinki.html)). Review of this protocol by the Institutional Review Board (IRB)/Ethics Committee (EC), and the performance of all aspects of the study including acquisition of informed consent, must also be in accordance with the principles elaborated in the Declaration, as well as the ICH guidelines (Code of Federal Regulations (CFR), Title 21: Part 50 and Part 312). The principal investigator will be responsible for submitting documents to the IRB/EC, and obtaining written approval for the protocol prior to study initiation. The approval of both the protocol and the informed consent must specify the date of approval, protocol number and version, and amendment number. The principal investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or other circumstances that may result in added risk to participating patients.

### **11.2 Protocol Amendments**

Before starting the study, the protocol must be approved by the IRB/EC, the JHU Institutional Biosafety Committee (IBC), the FDA, and the Recombinant DNA Advisory Committee (RAC). Amendments to the protocol are subject to IRB approval before instituting. Any amendments made after IRB/EC approval is granted must be resubmitted to the IRB/EC for new approval.

### **11.3 Written Informed Consent**

Before obtaining consent, members of the study team must review the rationale for the treatment program with the patient. The discussion will review the alternatives available, the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. Should an adverse event occur, the provisions available to ensure medical intervention must also be reviewed. Why the risks are reasonable in relation to the anticipated benefits, incentives, or costs that will/may be incurred as a result of participating in the study, as well as the efforts to maintain confidentiality, should also be discussed with the patient.

Patients will be required to sign and date (in triplicate) a statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the IRB. The consent form should be submitted with the protocol for review and approval by the IRB/EC. The medical record should include a statement that written informed consent was obtained (and should document the date that it was obtained) before the patient is enrolled in the study. The original signed consent document will become part of the patient's medical record, a copy will be forwarded to the project manager pursuant to registration, and a copy will be sent home with each patient.

The consent form must include the following information:

- the nature and objectives, potential toxicities, and benefits of the intended study
- the length of therapy and follow-up required
- alternatives to the proposed therapy (including standard and investigational therapies)
- the name of the investigator(s) responsible for the protocol
- the right of the patient to accept or refuse treatment and to withdraw from participation in the study at any time

#### **11.4 Protection of Privacy**

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. After this discussion, they will be asked to sign a Notice of Privacy Practice research authorization/HIPAA form. This may be embedded within the informed consent document. The original signed documents will become part of the patient's medical records, and each patient will receive a copy of the signed documents. The use and disclosure of protected health information will be limited to the individuals described in the research authorization form. The research authorization form must be prepared by the principal investigator and approved by the IRB.

In compliance with US federal regulations, the investigator is required to permit representatives of the US Food and Drug Administration (FDA) or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws. Patients will be informed of the extent to which their confidential health information generated from this study may be disseminated to other parties. Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the investigator to obtain such permission in writing from the patient.

#### **11.5 Study Termination or Modification**

Adverse events and laboratory data from this trial will be assessed by the principal investigator and/or medical monitor on an ongoing basis. At least quarterly, data from the clinical database will be reviewed. The results of this review will be shared with all investigators and MacroGenics either in writing or as part of a teleconference. SAEs will be reviewed as they are reported to the principal investigator or project manager, and the medical monitor will make an assessment regarding the safety of continuing or modifying the study. This assessment will be shared with the investigators either in writing or as part of a teleconference. Should the assessment of either the principal investigator or the medical monitor be that the trial should be terminated, the study will then be closed to further accrual. Follow-up safety assessments will be performed for all patients who are taken out of the study prematurely.

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**APPENDIX A: PERFORMANCE STATUS CRITERIA**

<i>ECOG Performance Status Scale</i>		<i>Karnofsky Performance Scale</i>	
<b>Grade</b>	<b>Description</b>	<b>%</b>	<b>Description</b>
0	Normal activity. Fully active, able to continue all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease
		90	Able to carry on normal activity, minor signs or symptoms of disease
1	Symptoms, but ambulatory. Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature ( <i>e.g.</i> , light housework, office work).	80	Normal activity with effort, some signs or symptoms of disease
		70	Cares for self, unable to carry on normal activity or to do active work
2	In bed < 50% of the time. Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance but is able to care for most needs
		50	Requires considerable assistance and frequent medical care
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair > 50% of waking hours.	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled, cannot carry on any self-care, totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly
5	Dead	0	Dead

**APPENDIX B: COMMON TOXICITY CRITERIA, VERSION 4.0**

Adverse events will be described and graded using the NCI Common Toxicity Criteria (Version 4.0). A copy of this document can be downloaded from the CTEP website (<http://ctep.cancer.gov/forms>). All treatment areas must have a copy of this document, or must be able to access a copy.

In general, the grading system can be summarized as follows:

<b><i>Grade:</i></b>	<b><i>Severity:</i></b>	<b><i>Description:</i></b>
Grade 1	Mild	Transient or mild discomfort; generally non-progressive; no limitation in daily activities; no medical intervention required.
Grade 2	Moderate	Mild/moderate limitation in daily activities; some assistance may be required; no/minimal medical intervention required.
Grade 3	Severe	Marked limitation in daily activities; some assistance usually required; medical intervention is required.
Grade 4	Life-threatening	Extreme limitation in daily activities; major assistance required; significant medical intervention required.
Grade 5	Death	Death related to an adverse event.

**APPENDIX C: SERIOUS ADVERSE EVENT (SAE) REPORTING FORM**

**Please notify Dr. Emmanuel Antonarakis within 24 hours**

<b>Protocol Title:</b>	Neoadjuvant Enoblituzumab (MGA271) in Men with Localized High-Risk Prostate Cancer – a Pilot and Biomarker Study		
<b>Protocol Number:</b>	<b>Principal Investigator:</b>	<b>Signature of PI:</b>	<b>Date:</b>
<b>Report Date:</b>	<b>Hospital Admission Date:</b>	<b>Date of Discovery of Event:</b>	<b>Report Type:</b> <input type="checkbox"/> Initial <input type="checkbox"/> Follow-up <input type="checkbox"/> Final Follow-up <input type="checkbox"/> Death <input type="checkbox"/> Addendum to:
<b>Section A: Subject Information</b>			
<b>Subject ID:</b>	<b>Subject Initial:</b>	<b>Subject Gender:</b> <input type="checkbox"/> Male <input type="checkbox"/> Female	
<b>Section B: Event Information</b>			
<b>Event diagnosis or symptoms:</b>	<b>Date of MGA271 Dose:</b>	<b>Action taken regarding the study drugs:</b> <input type="checkbox"/> None <input type="checkbox"/> Interrupted <input type="checkbox"/> Discontinued	
<b>Event Onset Date:</b>		<b>Event End Date:</b>	
<b>Relationship to:</b>	<b>MGA271</b>	<b>Underlying Disease</b>	
<b>Unrelated</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Probably Unrelated</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Possible Related</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

<b>Probably Related</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
<b>Definitely Related</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		
<b>Section C: Brief Description of the Event:</b>					
<b>Section D: Relevant Medical History</b>					
<b>Section E: Concomitant Drug (Not related to SAE)</b>					
<b>Name of the Drug</b>	<b>Start Date</b>	<b>Stop Date</b>	<b>Route</b>	<b>Dose</b>	<b>Frequency</b>
<b>Section F: Comments</b>					
<b>Additional Documents:</b> <input type="checkbox"/> Please specify					

**APPENDIX D: ABBREVIATIONS AND ACRONYMS**

ACLS	Advanced Cardiac Life Support
ADR	adverse drug reaction
ADT	androgen deprivation therapy
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
AR	androgen receptor
AST	aspartate aminotransferase
BCG	bacillus Calmette-Guerin vaccine
bid	bis in die (twice a day)
BP	blood pressure
BSA	body surface area
BUN	blood urea nitrogen
°C	degrees Celsius
Ca <sup>++</sup>	calcium
CBC	complete blood count
CD4 <sup>+</sup> T cells	cluster determinant 4–positive T lymphocytes
CD8 <sup>+</sup> T cells	cluster determinant 8–positive T lymphocytes
CFR	Code of Federal Regulations
CI	confidence interval
Cl <sup>-</sup>	chloride
cm	centimeter
COPD	chronic obstructive pulmonary disease
CR	complete response
CRO	Clinical Research Office
CRF	case report form
CRPC	castration resistant prostate cancer
CRRMC	Clinical Research Review and Monitoring Committee
CT	computerized tomography
CTL	Cell Therapy Laboratory
CTLA-4	cytotoxic T lymphocyte-associated antigen 4
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DAB	3,3'-diaminobenzidine tetrahydrochloride

dL	deciliter
DLT	dose-limiting toxicity
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DPBS	Dubelcco's phosphate buffered saline
DSMC	data and safety monitoring committee
DTH	delayed-type hypersensitivity
EC	ethics committee
ECOG	Eastern Cooperative Oncology Group
EMLA	eutectic mixture of local anesthetics
FDA	Food and Drug Administration
GCP	good clinical practice
G-CSF	granulocyte-colony stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GnRH	gonadotropin-releasing hormone
HA	hemagglutinin
HIPAA	Health Insurance Portability and Accountability Act
HR	heart rate
HRPC	hormone-refractory prostate cancer
HSA	human serum albumin
HSB	hue-saturation-brightness
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
IHC	Immunohistochemistry
ID	intra-dermal
IND	investigational new drug
INR	international normalized ratio
IQR	interquartile range
IRB	Institutional Review Board
IV	intravenous
JHMI	Johns Hopkins Medical Institutions
K <sup>+</sup>	potassium
LDH	lactate dehydrogenase
LHRH	luteinizing hormone releasing hormone
LNCaP	AR-positive human prostate cancer cell line derived from a lymph node metastasis
LOI	letter of intent
MedDRA	Medical Dictionary for Regulatory Activities

MTD	maximum tolerated dose
NA	not applicable
N/A	not available
NCI	National Cancer Institute
OR	operating room
PI	principal investigator
PO	per os (by mouth)
PSA	prostate-specific antigen
PSMA	prostate-specific membrane antigen
PT	prothrombin time
PTT	partial thromboplastin time
qd	quaque die (every day)
RP	radical prostatectomy
RT	room temperature
SAE	serious adverse event
SC	Subcutaneous
SD	standard deviation
SKCCC	Sidney Kimmel Comprehensive Cancer Center
TdT	terminal deoxynucleotidyl transferase
tid	ter in die (3 times a day)
TMA	tissue microarray
TRAMP	transgenic adenocarcinoma of the mouse prostate (mouse model)
T <sub>regs</sub>	regulatory T lymphocytes
TUNEL	TdT-mediated deoxy uridine triphosphate (UTP) nick end-labeling assay
ULN	upper limit of normal
USP	United States Pharmacopeia (sterile, hypotonic, nonpyrogenic water for injection)
WBC	white blood cell

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## **APPENDIX E: DATA AND SAFETY MONITORING PLAN (DSMP)**

This is a DSMP Level II study under the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data Safety Monitoring Plan (DSMP, Version 5.0: 12/6/2012, NCI Approval Date: 12/11/2012).

Data Monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by the Principal Investigator and externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

Additionally, scheduled meetings will take place monthly and will include the protocol principal investigator, research nurse, data manager, and, when appropriate, the collaborators, sub-investigators, and biostatistician involved with the conduct of the protocol.

During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

## APPENDIX F: SPECIMEN COLLECTION AND SHIPPING

### 1. Sera for Immunoassays

At each time point (Pre-treatment, Radical Prostatectomy, and Follow-up), the following research blood samples should be collected and processed as outlined below:

- Draw approximately 10 mL of peripheral blood into SST (tiger top, i.e. BD Vacutainer Cat #367985) tubes, each containing  $\geq 5$  mL of blood per vacutainer.
- Allow blood to coagulate for 20 minutes, then centrifuge at 25°C, 1500 x g (2700-3000 rpm), for 15 minutes.
- Pipette the serum into 10 cryotubes (about 0.5 mL/tube).
- Store cryotubes frozen, below -20°C (-70°C preferred), until the time of analysis.

Please [Ping “Vaccine Team” \(pager 410-283-0693\)](#) and the Immune Core will be notified to pick up the sample after draw.

### 2. Peripheral blood lymphocytes (PBLs)

At each time point (Treatment Day 1, Treatment Day 36, and Follow-up day 30), the following research blood samples should be collected and processed as outlined below:

- Draw approximately 100 mL of peripheral blood into two 50-mL heparinized conical tubes.
- PBLs will be prepared by Ficoll-Hypaque density gradient centrifugation according to standard protocols
- Samples will be cryopreserved in a liquid nitrogen freezer at -140°C for further batched analyses.

Please [Ping “Vaccine Team” \(pager 410-283-0693\)](#) and the Immune Core will be notified to pick up the sample after draw.

### 3. Radical Prostatectomy Specimen

All pathologic specimens will be handled in routine fashion by the operating room (OR) staff, except that as soon as the specimen is removed the OR nurse will directly page a tissue harvesting technician that is part of the Brady Urological Research Institute Prostate Specimen Repository Team. Accessioning of pathology specimens will be coordinated in the OR areas by the Departments of Urology and Pathology. A study technician will be available to the pathologist receiving the specimen to assure that the tissue is handled appropriately for the intended bioassays. At the time of harvesting, the pathologist will apply ink and cut the prostate specimen in transverse sections. The specimen will be fixed in 10% phosphate buffered formalin, and tissue blocks will be paraffin-embedded and sectioned at 4  $\mu$ m thickness for routine histologic evaluation and for immunohistochemical determinations. Fixation should occur as soon as possible after operative removal of the prostate, and ideally within 30-60 minutes. Assessment of index tumors for Gleason grade, nodal involvement, and pathologic staging will be conducted in usual fashion and will be provided to the

patient for prognostic information. After the pathology report is available, a database will be established and all information from the pathology reports of all samples will be included. Tumor blocks and/or additional unstained slides will be collected for study-specific quantitative immunohistochemical evaluations.

Tissue microarrays (TMAs) from formalin-fixed tumor samples will be analyzed for the degree of tumor apoptosis using immunohistochemical staining for activated caspase 3 (Marcelli et al 2000), or by using the method of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (i.e. TUNEL assay) (Furuya et al 1995). The in situ Cell Death Detection Kit (Roche, Indianapolis, IN) may be used to perform TUNEL reactions. Quantification of staining percentage will be achieved using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue. Analyses will be performed in the laboratory of Dr Angelo DeMarzo.

The contact information for the DeMarzo laboratory is listed below:

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